DEVELOPMENT OF BROAD-ACTING ENTEROSORBENTS FOR THE MITIGATION OF TOXIN EXPOSURES DURING OUTBREAKS AND EMERGENCIES

A Dissertation

by

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ABSTRACT

People and animals can be unintentionally exposed to mixtures of hazardous mycotoxins and environmental chemicals following natural and man-made disasters through contaminated food, feed supplies and drinking water. To develop effective sorbents to sequester and detoxify mixtures of toxins, we have amended parent calcium and sodium montmorillonite clays with the natural nutrients L-carnitine and choline to enhance the lipophilicity of the clay surfaces. Additionally, montmorillonite clays were processed and activated by sulfuric acid to simulate activated carbon's porosity and surface area. Clay-based enterosorbent therapy has been reported in previous animal and human clinical trials confirming the safety and efficacy of montmorillonite clay inclusion in diets.

In this study, isotherm analyses showed that carnitine/choline amended montmorillonite clays and acid processed montmorillonite clays (APMs) were effective sorbents for chemicals with diverse structures and properties, including important mycotoxins, i.e. aflatoxin and zearalenone (ZEN) and the hazardous environmental chemicals, i.e. benzo[a]pyrene (BaP), aldicarb, glyphosate and polychlorinated biphenyls (PCBs). A hydra bioassay developed in our laboratory further confirmed the safety of clay inclusion in diets and the protective effects against individual toxins. Besides decreased expansibility in water, higher surface areas, lower levels of trace metals and enhanced binding capacities of the newly developed clays, the enthalpy results suggested that the

adsorption reaction can be classified as a chemisorption, involving tight binding of toxins to clay surfaces. This is the first report of a sorbent (other than activated carbon) with high binding efficacy for these toxins. Also, protection of hydra against a mycotoxin mixture and a PCB mixture indicated that APMs were able to adsorb mixtures of toxins due to high capacities. Based on our results, the carnitine/choline amended montmorillonites and APM clays can be delivered in water, capsules, food, vitamins, etc. as broad-acting toxin enterosorbents for the mitigation of mixtures of hazardous mycotoxins and environmental chemicals.

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Contributors

This work was supervised by a dissertation committee consisting of Professors Timothy Phillips (Department of Veterinary Integrative Biosciences), Roger Harvey (Department of Veterinary Integrative Biosciences), Yanan Tian (Department of Veterinary Physiology & Pharmacology) and Natalie Johnson (Department of Veterinary Physiology & Pharmacology).

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1. INTRODUCTION

Public health is closely related to the external living environment and the risk of human exposures to hazardous chemicals. The consumption of food and water contaminated with environmental chemicals can frequently occur at the site of natural disasters. Food-borne mycotoxins, can be unintentionally ingested by humans and animals during extended periods of heat and drought (Sanders et al., 1984). Mycotoxins are hazardous secondary metabolites produced by various fungi. Among the mycotoxins, aflatoxin and zearalenone are most commonly found in food such as cereal crops including corn, barley, oats and wheat, and they can produce significant adverse effects on agriculture and health (Grant and Phillips, 1998; Lemke and Phillips, 1998).

Aflatoxins are known human carcinogens and common contaminants of corn, peanuts, cottonseed and groundnuts (Cotty, 1991). Historically, aflatoxins have been of significant interest in the areas of food safety and public health in developing countries. However, due to ongoing global climate change, aflatoxins are of increasing concern in an extended "hot zone" between 40° north and south of the equator including parts of the developed world, such as North America and Europe (Cotty and Jaime-Garcia, 2007). It has been postulated that contamination has become widespread in areas (such as the Midwest) that were previously unaffected in the US (Cotty and Jaime-Garcia, 2007). Among the 16 naturally occurring congeners of aflatoxin, aflatoxin B_1 (AfB_1) is one of four secondary metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus.* Importantly, it is the most prevalent and most toxic of the aflatoxins.

Zearalenone, a resorcylic acid lactone, also known as F-2 toxin, is a nonsteroidal estrogenic mycotoxin produced by numerous species of *Fusarium*. As a result, zearalenone is found in a number of cereal crops and their derived food products (Kuiper-Goodman et al., 1987). Zearalenone is rapidly absorbed after oral administration. Although the degree of absorption is difficult to measure owing to its rapid biliary excretion, it appears to be extensively absorbed in rats, rabbits and humans (Kuiper-Goodman et al., 1987). Both deoxynivalenol (another important mycotoxin) and zearalenone from toxic *Fusaria* have been linked to scabby grain toxicoses in the US, China, Japan, and Australia with symptoms including nausea, vomiting and diarrhea.

Other than heat and droughts, natural and man-made disasters (such as hurricanes and floods) can significantly mobilize environmental chemical contaminants, expose humans and animals to contaminated soil/sediment and threaten the safety of municipal drinking water and food sources. A major challenge associated with these disasters and emergencies is the protection of: 1) vulnerable communities and neighborhoods, 2) first responders, and 3) those involved in management and cleanup of contaminated sites. Multiple classes of chemicals have been prioritized by the Agency for Toxic Substances and Disease Registry (ATSDR) as important chemicals, including various polycyclic aromatic hydrocarbons (PAHs), pesticides, metals, organic solvents, plasticizers, etc. (ATSDR, 2015).

In this study, benzo[a]pyrene (BaP), aldicarb, glyphosate and PCBs were selected to represent environmental contaminants based on their toxicities and extensive distribution. BaP is a well-known environmental pollutant and a human and animal

carcinogen (Wong et al., 2017). BaP is commonly found in contaminated water and sediment after natural disasters. The Environmental Protection Agency (EPA) reported that BaP was detected at high contamination levels that exceed EPA's excess lifetime cancer risk range after Hurricane Katrina in 2005 and Hurricane Harvey in 2017 (apnews.com; epa.gov). Besides natural disasters, significant amounts of BaP were found in the dust and smoke after the World Trade Center disaster in downtown New York City (Xu et al., 2014).

Aldicarb is an acutely toxic insecticide, ascaricide, and nematicide that belongs to the carbamate class. The toxicity of carbamate insecticides, as well as organophosphorus compounds (i.e. glyphosate), is due to the inhibition of the enzyme acetylcholinesterase (Bertrand and Bertrand, 1991). Exposure to aldicarb can stimulate lipid peroxidation and paralyze the respiratory system (Yarsan et al., 1999). Aldicarb is one of the major pesticides found in water and sediment samples in the US and Canada, including groundwater and drinking water in New York and Wisconsin. Importantly, 50% of the concentration in New York groundwater was above the state standard of 7 ppb; 0.9% of samples contained aldicarb at concentrations above 100 ppb (Jones and Marquardt, 1987).

Glyphosate is one of the most commonly used organophosphorus herbicides to control weeds. An important factor contributing to the dominant use of glyphosate is the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Its mode of action is by inhibiting enzymes involved in the synthesis of three amino acids: tyrosine, tryptophan and phenylalanine. The risk of

occupational exposure to glyphosate is increased in agricultural workers and the toxin can be detected in blood and urine samples from those who are highly exposed to glyphosate (Jauhiainen et al., 1991).

Polychlorinated biphenyls (PCBs) represent a family of toxic organic chemicals consisting of 209 congeners. PCBs were widely used as lubricants and fluids in electrical equipment and other applications until concerns about their toxicity and potential hazards from environmental exposures were reported in the 1970s. However, their physical and chemical properties such as low vapor pressure, low aqueous solubility, and inertness to water, acid, alkali and heat result in their extreme persistence in the environment, which can lead to their bioaccumulation and sustainability in food chains, especially fish and aquatic species (Carey et al., 1976). Fish (low in hazardous contaminants) are an important part of a healthy diet, however, limiting consumption of certain fish and seafood due to elevated PCB levels is widely recommended. Statewide advisories also urge people to limit their consumption of all fish and shellfish from freshwater or coastal areas during flooding seasons and disasters.

Since ubiquitous toxin contamination of food and water occurs worldwide, methods to decrease human and animal exposures are critically needed. A novel strategy to ameliorate toxin exposure is the use of clays in the diet to decrease exposure and bioavailability from the gastrointestinal tract. Therapeutic clays that can tightly bind toxins would significantly decrease the toxicity and carcinogenicity associated with toxin exposures. Despite the efficacy of parent montmorillonite clay for aflatoxin mitigation, it is limited in its ability to bind other mycotoxins, environmental chemicals and mixtures

of toxins. To develop broad-acting sorbents, we amended parent montmorillonite clays with positively charged nutrients such as L-carnitine and choline to increase the hydrophobicity of clay interlayer surfaces. Also, both calcium and sodium montmorillonite clays were treated with sulfuric acid to produce high surface area and porosities, similar to activated carbon materials. The final reaction product of the acid treated clay is an amorphous silica structure with high reactivity and catalytic activity (Tyagi et al., 2006). The main novelty in this study is the fact that we can use these carbon-like, porous montmorillonite clays and nutrient amended organophilic montmorillonite clays as broad-acting, therapeutic enterosorbents for individual toxins and/or mixtures of mycotoxins and environmental toxins during emergencies and outbreaks. Moreover, the parent montmorillonite clays have been shown to be safe for human and animal consumption based on numerous interventions in animals and human clinical trials in the US and Africa.

This study was aimed at developing broad-acting sorbents for binding individual chemicals and chemical mixtures by: 1) Activating montmorillonite clays with sulfuric acid to create a highly porous sorbent with high surface area, and 2) Synthesizing hydrophobic interlamellar surfaces through amendment with L-carnitine and choline. We investigated the binding parameters of the new clays using equilibrium isotherms, thermodynamic and dosimetry studies. We also used the adult hydra bioassay to predict the ability of clays to prevent the adverse effects of diverse mycotoxins, environmental toxins and mixtures. Multi-dynamics simulations, computational chemistry and computational modeling were used to delineate binding mechanisms and confirm *in vitro*

and *in vivo* results. The inclusion of optimal sorbents in human and animal diets will be act as a protective measure to minimize unintended exposures and decrease the bioavailability of mycotoxin and environmental toxin contaminants during outbreaks, emergencies and disasters.

1.1 Mycotoxins

1.1.1 Aflatoxin

1.1.1.1 Source of aflatoxin contamination

Aflatoxins are fungal toxins that are members of a larger family of mycotoxins. Mycotoxins are structurally diverse chemical compounds produced by fungi, which have been strongly implicated as precursors of toxicity and carcinogenicity in humans and animals for centuries. The primary route of exposure is ingestion, however dermal or inhalation exposure may also occur. Due to their frequent occurrence in food and feed, mycotoxins have the potential to increase economic risks and adversely affect the health of humans and animals.

Of approximately 300 naturally occurring mycotoxins, aflatoxin is the most toxic and therefore the most widely studied. Aflatoxins are largely produced by the common fungi *Aspergillus flavus* and the closely related species *A. parasiticus*. Aflatoxins are known human and animal carcinogens and common contaminants of important commodities such as corn and groundnuts. It was discovered that the aflatoxin metabolites consist of four major congeners designated as B_1, B_2, G_1, and G_2 (Figure 1) based on their fluorescence and R_f values from thin-layer chromatography. Among the naturally occurring aflatoxins, aflatoxin B_1 (AfB_1) is the most toxic and commonly detected in

various food and feed products. *A. flavus* produces mostly B aflatoxins, while *A. parasiticus* produces both B and G aflatoxins (Diener et al., 1987; Klick and Pitt, 1988). Generally, *A. flavus* and *A. parasiticus* fungi produce aflatoxins when the temperatures are between 24°C and 35°C, and will contaminate many commodities if the moisture content exceeds 7% (10% with ventilation) (Williams et al., 2004). The amount of contamination varies with climate, both temporally and spatially. This becomes increasingly important as droughts become more frequent and persistent and global temperatures rise. What used to be considered the hot zone for aflatoxin contamination, 20° north and south of the equator, may now extend into areas such as the southern half of the US and Europe (at 40^{o}).

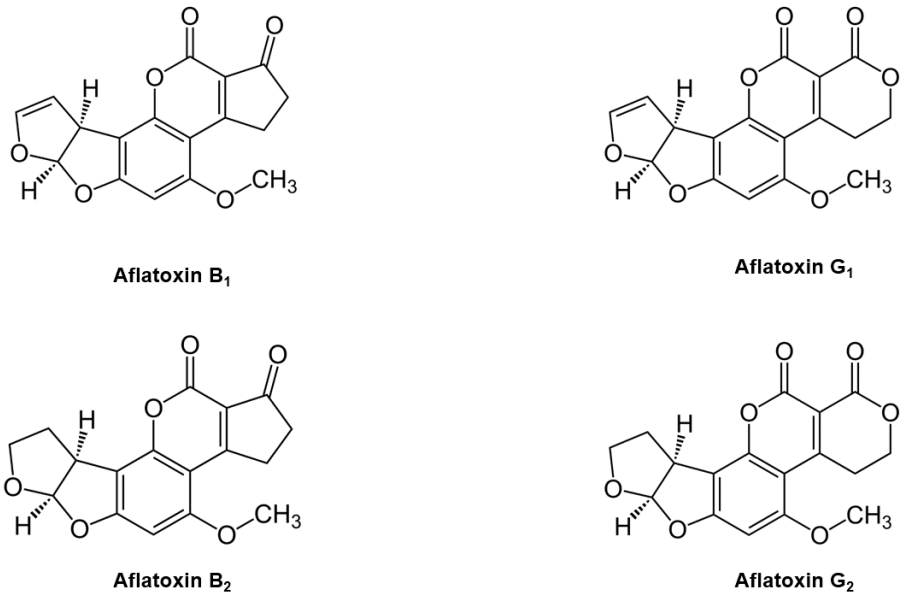

Figure 1. Chemical structures of the four naturally occurring aflatoxins B_1, B_2, G_1, and G_2. Aflatoxins are produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* fungi, and their nomenclature denotes a characteristic fluorescence emission under UV light, that is, (B) blue and (G) green fluorescence.

1.1.1.2 Toxicity

Due to strong epidemiological findings, the International Agency for Research on Cancer (IARC) classified AfB_1 as a Group 1 human carcinogen, following multiple studies in populations with high hepatocellular carcinoma incidence caused by AfB_1 (IARC, 1993; 2002).

Aflatoxin toxicity principally leads to structural and functional damage to the liver. Acute aflatoxin poisoning, or aflatoxicosis, in humans has been observed in multiple outbreaks and is characterized by vomiting, abdominal pain, pulmonary edema, and fatty infiltration and necrosis of the liver. Two forms of aflatoxicosis have been identified: the first is acute severe intoxication, which results in direct liver damage and subsequent illness or death, and the second is chronic sub-symptomatic exposure (Williams et al., 2004). Depending on the dose and duration of the exposure, aflatoxin toxicity may lead to acute illness and death, usually through liver cirrhosis; nutritional and immunologic consequences; and cumulative effects including cancer.

One of the most devastating outbreaks of aflatoxicosis occurred in the winter through early summer of 2004 in eastern Kenya (Azziz-Baumgartner et al., 2005). This outbreak resulted in 317 cases and 125 deaths. Health officials sampled maize from the affected area and the measured AfB_1 concentrations were 220 times greater than the 20 ppb limit for food suggested by Kenyan authorities. Early symptoms of aflatoxicosis include anorexia, malaise, low-grade fever and progress to acute hepatitis with vomiting, abdominal pain, and death (Etzel, 2002), which supported findings from a large body of animal work previously conducted. The first sign of exposure in all animal species is

decreased growth and loss of appetite. This initial observation led to the early studies demonstrating the role of aflatoxin in nutritional modulation, growth suppression, and immune system impairment.

Extensive research in West Africa has demonstrated a significant role for aflatoxin in growth stunting whereas, dietary insufficiency and infectious disease only explain about half of the restricted growth (Turner, 2013). In laboratory and domestic animals, chronic exposure to aflatoxins impairs immunity and interferes with protein metabolism and multiple micronutrients that are critical to health (Rogers, 1993). Importantly, children who are stunted often develop long-term developmental and cognitive problems, and are more vulnerable to infectious diseases (Ricci et al., 2006).

AfB_1 acts as a "force multiplier" synergizing the adverse effects of microbial pathogens and other agents or factors detrimental to health including immune suppression (Monson et al., 2015). The effects of aflatoxin on the immune system have been well demonstrated in a number of animal species as reviewed by Bondy and Pestka (2000). The main target of such studies have been cell-mediated immune responses (Ali et al., 1994; Bondy and Pestka, 2000; Neiger et al., 1994). As previously implied, growth faltering and micronutrient deficiencies are associated with decreased immune and non-immune host defenses that increase susceptibility to infectious diseases which contributes to the term immunotoxin (Wild, 2007).

Aflatoxins have also been shown to have an effect on zinc and selenium concentrations. Importantly, these minerals are essential for healthy immune systems. Specifically, zinc is required to activate a thymic hormone, thymulin (ZnFTS), which is

responsible for cell-mediated immunity (Mocchegiani et al., 1998). Intestinal malabsorption occurs in piglets from aflatoxin-exposed sows with defects related to a reduced zinc intestinal absorption (Miller et al., 1981).

Susceptibility to aflatoxin is greatest in the young, and there are significant differences between and within species, sexes (in terms of concentrations of testosterone), and nutritional factors (Pier, 1985).

1.1.1.3 Mitigation of aflatoxin exposure

Innovative strategies that significantly diminish the bioavailability of aflatoxins and mitigate human and animal exposures from contaminated food and feed have been developed. Based on the extant scientific literature, some of these approaches are already in the stages of clinical intervention and translation. Studies describing materials that tightly adsorb aflatoxins onto internal and/or external surfaces interfering with toxin uptake and bioavailability have recently been reviewed. Extensive studies with calcium montmorillonite clay (NovaSil, or NS) and dietary chlorophyllin in humans and animals indicate that these interventions are approaching implementation, but still require further clinical evaluation in the field to delineate the effects of dose and time on efficacy and safety as well as acceptability (Phillips et al., 2002; Wild and Turner, 2002). Other aflatoxin sequestering materials with limited evidence of efficacy will require preclinical trials in animals to confirm safety followed by clinical intervention trials in humans prior to implementation. Before full-scale implementation, all of these products should be rigorously evaluated *in vitro* and *in vivo*, and should meet the following criteria: (1) favorable thermodynamic characteristics of aflatoxin sorption, (2) tolerable levels of

potential hazardous contaminants, (3) safety and efficacy in multiple animal species, (4) safety and efficacy in long-term studies, and (5) negligible interactions with vitamins, iron and zinc and other micronutrients. Based on these criteria, montmorillonite clays are among the most thoroughly characterized sorbent materials and have produced the only aflatoxin intervention trials in humans (Phillips et al., 2019). Therefore, the use of montmorillonite clay has demonstrated potential application for the mitigation of aflatoxin exposure in animals and humans.

1.1.2 Zearalenone

1.1.2.1 Source of zearalenone contamination

Similar to aflatoxin, zearalenone is a fungal toxin, or mycotoxin, mainly produced by *Fusarium graminearum* on various cereal crops (Figure 2). The chemical name for zearalenone is 6-(10-hydroxy-6-oxo-trans-1-undecyl)-beta-resorcyclic acid lactone (Marasas and Nelson, 1987). Zearalenone can be detected by UV/visible scanning spectrometry with a distinct UV spectrum for absorbance at wavelengths of 236 nm (29,700), 274 nm (13,909) and 316 nm (6,202) (Cole and Cox, 1981).

Zearalenone production in crops is favored by low temperatures (6ºC to 18ºC) and grain moisture content of greater than 23%. Tanaka et al. conducted a comprehensive survey and found that half of the crops collected worldwide were positive for zearalenone. Contamination of barley was equal to 73% of samples, with 28% in corn samples and 43% in oats. Since these grains are frequently included in animal feedstuffs, this finding suggests a high risk of zearalenone exposure in livestock.

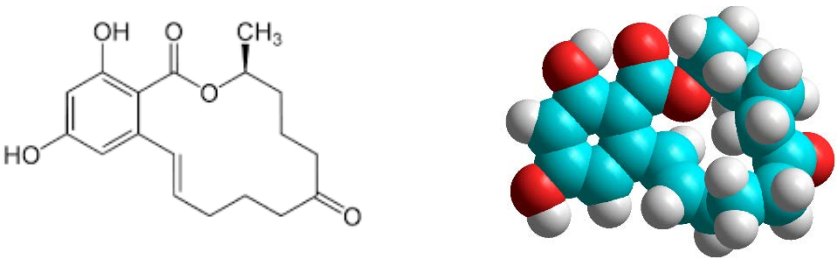

Figure 2. Chemical structure and molecular model of zearalenone.

1.1.2.2 Toxicity

Due to the rapid biotransformation and excretion of zearalenone in animals, it is most toxic by intraperitoneal injection (Creppy, 2002). However, dietary intake from meat and meat products is a major exposure route for zearalenone due to its use in animals as an anabolic steroid. Considering the mean levels of zearalenone in the principal foods and their consumption, the average daily intakes of zearalenone ranged among adults from 0.8 to 29 ng/kg bw, while small children had the highest average daily intakes ranging from 6 to 55 ng/kg bw/day (Minervini et al., 2005).

Zearalenone cytotoxicity seriously affects reproduction (Kiang et al., 1978; Mehmood et al., 2000; Nikov et al., 2000), immunity (Abbes et al., 2006a, b; Luongo et al., 2006), endocrine activities (Mueller et al., 2004), and inheritance (Kouadio et al., 2005) of animals, and there is currently no effective antidote for this toxin (Cortinovis et al., 2013). Swine are the most sensitive domestic species, followed by ruminants, while birds are the most resistant species (Kuiper-Goodman et al., 1987; Olsen et al., 1987).

Recent studies have demonstrated the potential for zearalenone to stimulate growth of human breast cancer cells acting through the estrogen receptor (Ahamed et al., 2001;

Mayr, 1988). Zearalenone has also been shown to be genotoxic and to induce DNA-adduct formation, DNA fragmentation and micronuclei production (Abid-Essefi et al., 2003, Abid-Essefi et al., 2004; Lioi et al., 2004).

Of all the maturity stages, the pre-pubertal stage in porcine is the most sensitive to zearalenone treatment (Bohm, 1992). During pregnancy, zearalenone reduces embryonic survival when administered above a threshold and sometimes decreases fetal weight (D'Mello et al., 1999). Zearalenone may affect the uterus by decreasing luteinizing hormone and progesterone secretion and by altering the morphology of uterine tissues (Etienne and Dourmad, 1994; Zhang et al., 2018).

1.1.2.3 Mitigation of zearalenone exposure

Detoxification strategies for contaminated foods and feeds to reduce or eliminate the toxic effects of zearalenone by chemical, physical, and biological methods are crucial to improve food safety, prevent economic losses, and reclaim contaminated products. Previous published biological processes to degrade and adsorb zearalenone include: Mannan-oligosaccharides from the cell wall of *S. cerevisiae 1026* (Devegowda et al., 1996), bacteria culture (Magharaj et al., 1997; Mokoena et al., 2002), ruminant protozoa (Kiessling et al., 1984), etc. Also, Ramos et al. (1996) reported that bentonite, sepiolite, Mg trisilicate, cholestyramine, crospovidone can all adsorb zearalenone. Other physical detoxification methods that have been reported include using montmorillonite and modified montmorillonite with cetylpyridinium or hexadecyltrimethylammonium (Lemke et al., 1998) and activated carbon (Ryu et al., 1999). Beside biological and physical methods, chemical processes using ozone (McKenzie et al., 1997) and hydrogen peroxide

(Abdalla, 1997) were also reported to significantly degrade zearalenone. High concentrations of ozone were reported to degrade zearalenone at 15 s to a significantly less toxic form.

1.2 Superfund environmental chemicals

1.2.1 Benzo[a]pyrene

1.2.1.1 Source of benzo[a]pyrene contamination

Benzo[a]pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants formed during incomplete combustion or pyrolysis of organic material (Figure 3). These substances are found in air, water, soils and sediments, generally at trace levels except for near their sources. There is no quantitative relation between measured BaP and concentrations of any other PAHs; however, if BaP is found, other PAHs are usually present. Major sources of PAHs in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses, indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires (IARC, 2012). Oil and gas heating produce much lower quantities of PAHs.

PAHs are present in some foods and in a few pharmaceutical products based on coal tar products that are applied to the skin for the treatment of psoriasis. BaP has been observed to accumulate in marine organisms and plants which could indirectly cause human exposure through food consumption. Drinking water is estimated to account for only 0.1 to 0.3% of the total BaP ingested. Air is estimated to contribute about 0.9% of

the total exposure. The greatest source of exposure is through foods, which contribute 99% (WHO, 1984). Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high-temperature processing); bread, cereals and grains (at least in part from gas/flame-drying of grains); and vegetables grown in contaminated soils, or in areas with surface contamination from atmospheric PAH fall-out (Boström et al., 2002; IARC, 2010). In ambient air, BaP, as well as PAHs in general, is normally associated with fine particulates. In a recent review, it was reported that average atmospheric concentration of BaP in summer is 3.6 ng/m^3 (standard deviation 4.0). In winter, concentrations are higher (mean 7.1 ng/m^3; standard deviation 5.1), probably because of the contribution from industrial and domestic heating, which uses fossil fuels. Tobacco smoke contains high concentrations of PAHs (IARC, 2010). The amount of BaP per cigarette is between 18 and 50 ng, and total PAHs can amount to as much as 248 ng (Zedeck, 1980).

In addition to air, sediment and soil, PAHs may accumulate in marine organisms (EPA, 2014). Uptake of PAHs by marine organisms is dependent on the bioavailability of the PAHs (i.e. partitioning of the compound between sediment, water and food), as well as the physiology of the organism (Meador et al., 1995). The organism size, ingestion rate, growth rate, membrane permeability, ventilatory rate, gut residence time and osmoregulation are biological processes that influence the organism uptake of PAHs. For example, Baumard et al. (1999) observed higher concentrations of PAHs in mussels during sampling in March (870 ng g^{-1}) as compared to October (250 ng g^{-1}). Mytilus edulis has been shown to have higher feeding rates at the end of winter (Deslou-Paoli et al., 1987),

and therefore, the higher filtering rate in March induced a greater exposure to the PAHs in the water column. Environmental factors, such as temperature, oxygen content, pH and salinity can also influence the uptake of PAHs by marine organisms due to their effect on the bioavailability of the compounds. In addition, changes in the organism's behavior, seasonal rhythms, nutritional quality and stress can also influence PAH uptake.

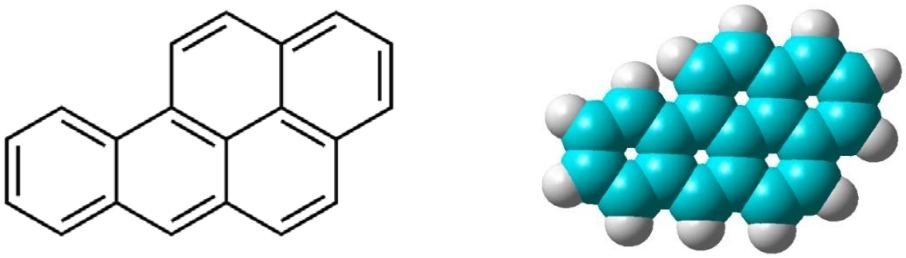

Figure 3. Chemical structure and molecular model of benzo[a]pyrene.

1.2.1.2 Toxicity

Benzo[a]pyrene is a carcinogen that induces tumors in many animal species. Some of the carcinogenicity studies include lung tumors in mice, rats, and hamsters; skin tumors in mice; liver tumors in mice; forestomach tumors in mice and hamsters; and mammary gland tumors in rats (Osborne and Crosby, 1987; IARC, 2010). In humans, occupational exposures to BaP-containing mixtures have been associated with a series of cancers, and thus it is classified as a carcinogen to humans (Group 1).

Studies on the mechanisms of action of BaP have been reviewed. BaP is absorbed following exposure by inhalation, oral, and dermal routes. The rate and extent of

absorption are dependent upon the exposure medium. The presence of BaP in body fat, blood, liver, and kidney and the presence of BaP metabolites in serum and excreta indicate wide tissue distribution. BaP metabolism occurs in essentially all tissues, with high metabolic capacity in the liver and significant metabolism in tissues at the portal of entry (lung, skin, and gastrointestinal [GI] tract) and in reproductive tissues. BaP is metabolized by both phase I and phase II enzymes. BaP-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on BaP-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of BaP-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of BaP. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1 (Eling et al., 1986; Shimada, 2006). CYP450s are inducible by BaP and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, leading to changes in gene transcription of CYPs and phase II enzymes. The primary route of elimination is in the feces, particularly following exposure by the inhalation route. Overall, BaP is eliminated quickly with a biological half-life of several hours (EPA, 2011).

Other than carcinogenicity and DNA damage, BaP is also known to cause proliferation, oxidative stress, and interference of signal transduction pathways involved in Ca^{2+} homeostasis and the regulation of epidermal growth factor. BaP is known to suppress humoral and cell-mediated immunity by altering the antigen and mitogen receptor signaling pathways. At high exposure levels, BaP activates genes involved in apoptosis in lymphoid cells (Burchiel and Luster, 2001; Oh et al., 2004). Experimental data demonstrate that, similar to most lipophilic compounds, BaP easily crosses the blood–

brain barrier, thereby gaining direct access to the central nervous system (Ramesh et al, 2001; Zhang et al., 2008). Neurological symptoms, such as autonomic dysregulation and short-term memory loss, have been reported in workers exposed occupationally to BaP.

1.2.1.3 Mitigation of benzo[a]pyrene exposure

In air, BaP is predominantly adsorbed to particulates, but may also exist as a vapor at high temperatures (HSDB, 2012). BaP vapor is eventually removed from the atmosphere by its photochemical oxidation and dry deposition on land or water. The conventional water treatment processes of alum coagulation, settling and sand filtration are capable of reducing the BaP concentration of surface waters to less than 0.001 mg/L, even if the influent concentration is high (Ontario Ministry of the Environment, 1985).

The adsorption or covalent attachment of BaP to natural solids, such as clays and humic materials can significantly affect the bioavailability, transport, biological activity and degradation of the compound in the environment (Pignatello and Xing, 1996). Weissenfels et al. (1992) and Erickson et al. (1993) observed a decrease in the mineralization of high molecular weight PAHs with increasing residence time in soils. This decrease in PAH degradation was attributed to the association of PAHs to soil organic matter. Such associations may result in a reduction in the rate and extent of PAH degradation due to the slowing of PAH desorption from soil organic matter into the soil aqueous phase (Guthrie and Pfaender, 1998). A number of mechanisms, such as those through van der Waals forces, chemical binding, cation bridging, H-bonding, ion exchange, covalent bonding or ligand exchange (Khan and Ivarson, 1982, Koskinen and

Harper, 1990), may be involved in the sorptive process; however, these mechanisms are not fully understood.

Adsorption of PAHs on carbonaceous adsorbents, in particular carbon nanomaterials and their modified forms, has been actively investigated in the past decades (Cortés-Arriagada, 2017; Lohmann et al., 2005; Wu et al, 2012; Van Noort et al., 2004). The carbon nanomaterials, including fullerenes, single- and multi-walled carbon nanotubes (CNTs), and graphene (Gr), have been demonstrated to have a high potential for adsorbing organic pollutants including PAHs (Yang et al., 2006; Al-Degs et al., 2008; Ncibi and Sillanpaa, 2015). Specifically, the adsorption characteristics of various PAHs onto Gr and its oxide surfaces have been widely studied under various conditions of pH, temperature or humic acid (HA) (Apul et al., 2013; Du et al., 2016; Perreault et al., 2015; Radian and Mishael, 2012; Sun et al., 2013).

Other than carbon materials, sediments have also been shown to be able to adsorb BaP in aquatic systems. Bowman et al. (2002) reported that high sediment concentration (with the existence of colloid) contributed to the high sorption coefficient. The sorption of BaP was also dependent on some of the particle properties, and the sorption coefficient was found to increase with the organic carbon content and specific surface area of sediment particles. The desorption of BaP from sediment was shown to be relatively rapid, with implications for the potential remobilization of BaP and similar compounds.

The ability of isolated bacteria, fungi and algae to degrade high molecular weight PAHs, such as BaP was investigated (Juhasz and Naidu, 2000). Although bioremediation is generally regarded as an economical remediation option for the clean-up of PAH-

contaminated soil, the successful application of this technology is restricted by the limited capacity of micro-organisms to degrade high molecular weight PAHs. Many bacteria, fungi and algae have the ability to degrade a range of low molecular weight PAHs, such as naphthalene, fluorene and phenanthrene, however, their activity towards PAHs containing five or more fused benzene rings, such as BaP, is limited.

The destruction of the carcinogen BaP by light is well known; Oxidation rates as high as 100% per hour of exposure are observed when less than 0.1 micrograms of BaP is coated inside quartz tubes and exposed to ozone or sunlight. The oxidation rates for BaP seem to depend very much on the surface area of exposure. The actual oxidation rates by sunlight and ozone can be enhanced by more efficient exposure of BaP when it is in the form of aerosols (Rajagopalan et al., 1983). Ottinger et al. (1999) reported that as little as 2 min of O_3 treatment afforded protection from BaP-induced mortality and toxicity (embryolethality and liver discoloration) in chicken embryos. In the hydra bioassay, no toxicity was observed in the adult hydra until the ozonolysis products were reconstituted 100-fold from their initial post-ozonolysis concentrations.

1.2.2 Aldicarb

1.2.2.1 Source of aldicarb contamination

Aldicarb, 2-methyl-2-(methylthio)propionaldehyde o-methylcarbamoyloxime, is an oxime carbamate insecticide manufactured by the Union Carbide Corporation and sold under the trade name Temik since 1965. Its molecular structure and model are shown in Figure 4. It is a soil-applied systemic pesticide used against certain insects, mites, and nematodes, and is applied below the soil surface for absorption by plant roots. Aldicarb

disperses through the soil with soil moisture and on release from the granule, is taken up by plant roots and translocated through the plant to provide protection against chewing and sucking insects and nematode damage. It is generally applied to the soil in the form of 5, 10, or 15% granules, and soil moisture is essential for the release of the toxicant. Uptake by plants is rapid. Aldicarb is currently registered for use on cotton, sugar beets, sugar cane (Louisiana only), potatoes, sweet potatoes, peanuts, oranges, pecans (Southeast only), dry beans, soybeans, and ornamental plants. Home and garden use are not permitted. The mode of aldicarb action is systemic, and exposure of pests to this active constituent affects the nervous system by inhibiting the activity of acetyl cholinesterase. Aldicarb is metabolically transformed to aldicarb sulfoxide and aldicarb sulfone. Aldicarb sulfoxide has similar toxicity to aldicarb; aldicarb sulfone, also known as aldoxycarb, is considerably less toxic (approximately 4% of that of aldicarb) (National Registration Authority, 2001). Discovery of aldicarb and its oxidative sulfoxide and sulfone metabolites in well and/or ground water in Florida, Wisconsin and New York, and accidental poisonings from ingesting contaminated watermelons and cucumbers in the South and West have spurred interest and concern about this pesticide (Risher et al., 1987).

Figure 4. Chemical structure and molecular model of aldicarb.

1.2.2.2 Toxicity

The National Academy of Science reports that the acute toxicity of aldicarb is probably the highest of any widely used insecticide (National Academy of Sciences, 1977) and EPA has classified aldicarb in the highest toxicity category and has defined a strict control for its delivery and use. In Brazil and the Caribbean islands, aldicarb is illegally used as a household rodenticide with a widespread risk of poisoning. The potential severity of acute toxic poisoning by aldicarb is evidenced by a reported incident, in which six cows became ill and two of the six died following the accidental spilling of Temik in a pasture. Chemical analysis for aldicarb in the rumen of one of the dead animals showed that a lethal dose of the pesticide was present. Milk produced by the surviving animals on the day of the poisoning and the next 6 successive days had to be destroyed to prevent possible human health risks. Symptoms reported for accidental or occupational poisoning (CDC, 1979; Goes et al., 1980; Sexton, 1996) and controlled aldicarb human exposure (Haines, 1971) have been cholinergic in nature and have spontaneously subsided, generally within a 6-h period. Clinical symptoms varying in severity with the amount of insecticide consumed and the age and general health of the exposed individual included dizziness, skeletal muscle weakness, epigastric cramping pain, diarrhea, excessive sweating, nausea, vomiting, nonreactive contracted pupils, blurred vision, dyspnea, and muscle fasciculation or convulsions. Several human poisoning incidents resulting from the ingestion of aldicarb contaminated cucumbers and watermelons have been reported in the literature. In 1990, a published report endeavored to estimate aldicarb dosages from four separate poisonings that occurred in the United States between 1978 and 1988. The poisonings (some with

clinical signs) occurred at estimated doses of 0.001 to 0.06 mg/kg bw based on self-reports of the amount of fruit consumed, averaged weights of the consumable portions, estimates of body weights by age and sex, and estimates of aldicarb sulfoxide determined in the fruit. In a paper published in 1987, after 140 people had become ill from eating cucumbers adulterated with a pesticide, the estimate dose of aldicarb ingested for 13 cases ranged between 0.01 and 0.03 mg/kg bw/day.

The primary mechanism of toxic action of aldicarb is cholinesterase inhibition. Carbamate insecticides are known to directly affect the enzyme acetylcholinesterase (AChE), which is associated with the outer surface of membranes. This results in a buildup of acetylcholine (ACh), which acts on the plasma membrane to produce the primary expression of neurotoxicity (Blum and Manzo, 1985). It is commonly accepted that carbamates interfere with the ability of AChE to break down the chemical transmitter ACh at synaptic and myoneural junctions, although the precise biochemical mechanism for this interaction remains an object of discussion. However, unlike the relatively irreversible anticholinesterase activity of the organophosphate pesticides, the carbamylation process which produces the anti-AChE action is quickly reversible. Aldicarb is readily absorbed through both the gut and the skin, but is rapidly metabolized and excreted in the urine almost completely within 24 h, with urine accounting for almost all of the excreted toxic and relatively nontoxic (oxime and nitrile) metabolites.

Although it is acutely toxic to humans and laboratory animals, aldicarb is not known to be carcinogenic, teratogenic, conclusively mutagenic, or to produce other long-

term adverse health effects. However, the potential for *in vivo* transformation to a potentially carcinogenic nitroso derivative is cause for concern.

1.2.2.3 Mitigation of aldicarb exposure

Aldicarb has been detected in ground water across the United States, based on the literature on sorption and transport. Leaching of organic compounds through the soil poses a serious threat to ground water. Sorption to soil is a critical factor in determining how rapidly a pesticide will leach through the vadose zone. Several standard batch adsorption studies were conducted in a variety of soils in order to determine the partitioning behavior of aldicarb and its toxic sulfoxide and sulfone metabolites. These studies were supported by standard column leaching studies with aldicarb and its toxic metabolites, and by a specialized leaching study designed to demonstrate upward movement under drying conditions. Movement of aldicarb through the soil profile was modelled at three field sites with sandy soils in the Bundaberg district. Standard batch equilibrium studies indicate that aldicarb is only weakly adsorbed by soils, and its oxidation products even less so. Aldicarb and its toxic metabolites share significant water solubility and tend to move with soil moisture through the soil. Low adsorption coefficients indicate that mobility in soils is high, and this has been confirmed in leaching experiments with soil columns. Simple model calculations identify aldicarb as a probable leacher.

From other studies on the sorption of aldicarb onto different soils, it can be concluded that the organic amendments increased soil organic matter (SOM) content and thus greatly affected aldicarb sorption-desorption processes. The adsorption isotherms for clay, litter compost and sludge compost were S-type, while that of calcareous soil, sandy

soil, animal manure and chicken manure were C-type. The composting process improved the characteristics of organic amendments and increased their sorption capacity for aldicarb. Adsorption of aldicarb onto soils with low SOM was found to conform to the Freundlich equation. In addition, the magnitude of adsorption was found to be in the order: clay soil > calcareous soil > sandy soil. This relationship is consistent with the organic matter content of the different soils. As characterized by affinity values, sorption of aldicarb on compost of sludge and litter was about 1.5-fold higher than that on manure of animals and chickens (El-Aswad, 2007). Aldicarb behaves as a nonionic organic compound in most solvents at a pH between 1.2 and 13.8. It is adsorbed primarily by SOM and to a lesser extent by clay minerals.

1.2.3 Glyphosate

1.2.3.1 Source of glyphosate contamination

Glyphosate (Figure 5A) is a phosphonoglycine non-selective herbicide, first registered for use by the EPA in 1974. Glyphosate was discovered by Monsanto under the trade name Roundup and it is extensively used as an herbicide to control weeds. One of the most important factors contributing to the dominant use of glyphosate is the introduction of transgenic, glyphosate-resistant crops in 1996. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. Glyphosate/glyphosate-resistant crop weed management offers significant environmental and other benefits over the technologies that it replaces (Duke and Powles, 2008). Its mode of action is by inhibiting enzymes involved in the synthesis of three amino acids: tyrosine, tryptophan and phenylalanine. Therefore,

it is only effective on actively growing plants and not effective as a pre-emergence herbicide.

The manufacture and major use of glyphosate have led to its direct release into the environment (EPA, 1993). Once glyphosate enters the environment, it has low potential for environmental bioavailability and is unlikely to bioaccumulate; the chemical is either degraded by microbial processes to Aminomethylphosphonic acid (AMPA) (Figure 5B) or inactivated by adsorption to soil (Shushkova et al., 2010; Smith and Oehme, 1992). Glyphosate adsorbs strongly to soil, and residues are expected to generally be immobile in soil, thus ground and surface water pollution is limited, therefore, leaching into groundwater is minimal (Borggaard and Gimsing, 2008; Smith and Oehme, 1992). However, glyphosate may enter surface waters due to its use in some aquatic environments or during disasters when its mobilization in the environment is enhanced. Volatilization of glyphosate is not an important fate process based on its low vapor pressure and ionic nature (Smith and Oehme, 1992). Glyphosate has been detected in air during spraying, and water and food as well.

The general population may be exposed to glyphosate by dermal contact with consumer products, crops, foliage, or soils containing residues of this chemical; ingestion of plants, crops, food, or water containing residues of this chemical; and inhalation of mist or spray during the use of products containing this chemical. As a result of its widespread usage, glyphosate is present at low levels in a wide range of foods (FAO and WHO, 2016). The greatest potential for exposure can be expected for people who use glyphosate products at home and for populations residing near agricultural areas and farms,

manufacturing and processing plants where glyphosate is produced or used, and hazardous waste disposal sites containing glyphosate.

Occupational forest workers, gardeners, grounds workers, and farmers are highly exposed to glyphosate (Jauhiainen, et al., 1991). Occupational exposure of glyphosate may occur via inhalation, dermal contact, and/or ocular contact during manufacture, transport, use, and disposal. Dermal contact appears to be the major route of exposure to glyphosate for people involved in its application. Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Other susceptible populations include young children, elders, women during pregnancy and lactation, and patients with compromised immunity. The general population is primarily exposed via the oral route. The maximum absorption from the gastrointestinal tract is estimated to be up to 40%. The amount of glyphosate detected in tissues is negligible, suggesting limited distribution and low tissue retention following dosing. Parent glyphosate is the principal form excreted in urine and feces (main excretion route). Elimination is essentially complete by 24 h, indicating glyphosate does not bioaccumulate (EPA, 2016).

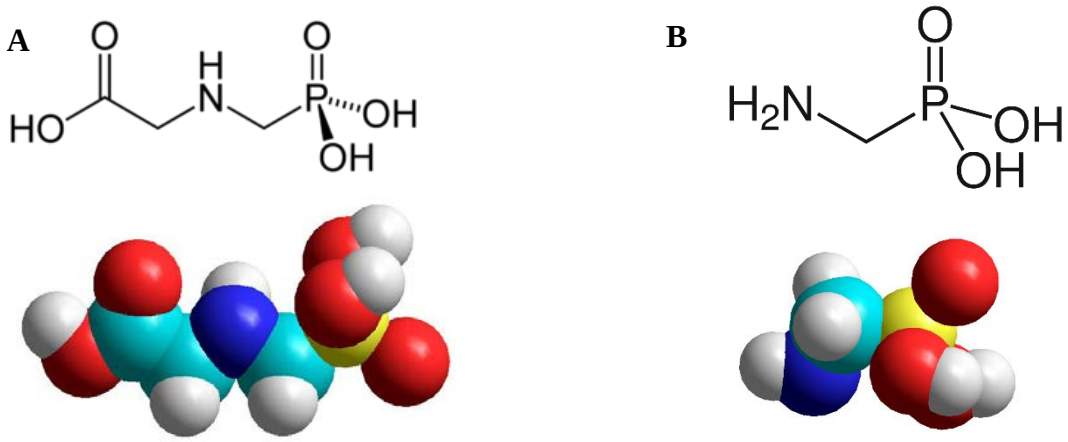

Figure 5. Chemical structures and molecular models of glyphosate (A) and its metabolite AMPA (B).

1.2.3.2 Toxicity

Glyphosate has been reported to be the least toxic pesticide to animals, with an LD_{50} for rats greater than 5 g kg^{-1} (Duke et al., 2003). It has low oral and dermal acute toxicity and its acute inhalation toxicity has been waived because glyphosate is nonvolatile and adequate inhalation studies with end-use products have shown low toxicity. Subchronic feeding studies at high dose levels showed gastrointestinal effects such as nausea, vomiting, abdominal pain, sore throat, and mucosal damage in the mouth and esophagus. Besides gastrointestinal symptoms, depressed body weight, increased liver weight, increased markers of liver toxicity, increased specific gravity of urine and decreased urinary pH were also observed in animal studies at high glyphosate exposure.

The problem with glyphosate exposure has become a contemporary and controversial issue. In 2015, it was classified as “probably carcinogenic in humans” by

IARC (International Agency for Research on Cancer) (IARC, 2015). From 2014 to 2016, several meta-analyses were conducted for lymphohematopoietic cancers. Case-control studies of occupational exposure in the US, Canada, and Sweden reported increased risks for non-Hodgkin lymphoma that persisted after adjustment for other pesticides (Guyton et al., 2015a, b). Eriksson et al. (2008) reported positive associations between glyphosate use and lymphocytic lymphoma (OR 2.56; 95% CI 1.17–5.60) and unspecified non-Hodgkin lymphoma (OR 5.29; 95% CI 1.60–17.50).

Importantly, the IARC conclusion was not confirmed by the EU assessment and the recent joint WHO/FAO evaluation. Both studies used additional evidence that glyphosate was not carcinogenic in rats, but they could not exclude the possibility that it was carcinogenic in mice at very high doses. This information was used in the risk assessment concluding that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet (European Food Safety Authority 2015a, b; Germany 2015). A majority of the follow-up studies did not report statistically significant associations between glyphosate use and many of the lymphohematopoietic cancer subtypes. Several other studies reported significant associations between glyphosate use and non-Hodgkin lymphoma, but these studies reported conflicting results depending on the statistical methods used, adjustment for confounders, or inclusion criteria.

1.2.3.3 Mitigation of glyphosate exposure

The Worker Protection Standard (WPS) for Agricultural Pesticides established an interim restricted entry interval (REI) of 12 h for glyphosate. The Agency has decided to retain this REI as a prudent measure to mitigate risks to workers. During the REI, workers

may reenter areas treated with glyphosate only in the few, narrow exceptions allowed in the WPS. The REI applies only to glyphosate uses within the scope of the WPS, so homeowner and commercial uses are not included (EPA, 1993).

Glyphosate has strong adsorption to most soils due to its ionic nature and is expected to bind to positively charged surfaces present in clay and soils. Adsorption occurs through hydrogen bonding, ion exchange, or complex formation between the phosphonate anion and/or the ammonium cation with active surfaces of minerals present in soils (Miles and Moye, 1988). Another study by Morillo et al. (2000) showed that glyphosate adsorption on soils with different characteristics was not related to their cation exchange capacity (CEC) and clay mineral content, but to the content of iron and aluminum amorphous oxides and organic matter. The presence of Cu in treatment solutions enhanced glyphosate adsorption, due to several reasons: glyphosate coordinates strongly to Cu, and Cu/glyphosate complexes seem to have higher ability to be adsorbed on the soil than free glyphosate; glyphosate adsorption can take place on sites where Cu was previously adsorbed, acting as a bridge between the soil and glyphosate; when Cu was present the solution pH decreased, and glyphosate adsorption increased, since lower pHs lead to the formation of glyphosate species with lower negative charge, which are adsorbed more easily on the negatively charged soil surfaces.

Although glyphosate is expected to adsorb strongly to soil particles and clay minerals, desorption may occur under certain conditions. It has been demonstrated that sorption decreases with increasing soil pH, increasing concentrations of inorganic soil phosphate due to competition to the same binding sites, and decreasing mineral

concentrations (Glass, 1987; Piccola et al., 1994; Plimmer et al., 2004; Smith and Oehme, 1992; Sprankle, 1975).

The major degradation product, AMPA, also binds to soils and may be more mobile than glyphosate (Duke and Powles, 2008; IPCS, 1994). The adsorption and desorption of both glyphosate and its metabolite AMPA were examined by Gerritse et al. (1996) using five soil types. The strongest adsorption occurred in the soil with the highest iron and aluminum content. The weakest adsorption occurred in the soil with the highest organic content. These results indicate that glyphosate has a notable affinity towards some soils, particularly with lower pH values and greater mineral content, and desorption occurs under certain environmental conditions especially as pH values increase and mineral concentrations decrease.

1.2.4 Polychlorinated biphenyls

1.2.4.1 Source of polychlorinated biphenyls contamination

Polychlorinated biphenyls (PCBs) are chlorinated aromatic hydrocarbons which were discovered in 1881 (Figure 6A). The physical and chemical properties of PCBs, such as stability, resistance to degradation, oxidation, and chemical agents such as acids and bases, low vapor pressures and fire resistance (Clayton and Clayton, 1994) result in their widespread used as coolants and lubricants in transformers, capacitors, and other electrical equipment.

There are four major routes by which PCBs enter the environment: industrial accidents or discharges, incomplete destruction of PCB containing products, natural weathering of PCB containing products, and leaking from landfills (Clayton and Clayton,

1994). Once in the environment, PCBs do not readily break down and therefore may remain for very long periods of time. They can easily cycle between air, water, and soil. In water, PCBs may be transported by currents, attach to bottom sediment or particles in the water, and evaporate into air. Sedimentation testing during the early 1970's in Lake Erie (1971), Lake St. Clair (1970 and 1974), and the Detroit River (1974) revealed residues of PCBs were 3 times higher than those of all organochlorine insecticides. These levels have decreased since the restrictions of PCBs (Brooks, 1969). Concentrations of PCBs in subsurface soil at a Superfund site have been as high as 750 ppm. Sediments that contain PCBs can also release the PCBs into the surrounding water. People who live near hazardous waste sites may be exposed to PCBs by consuming PCB contaminated fish and animals, by breathing PCBs in air, or by drinking PCB-contaminated well water. Adults and children may come into contact with PCBs when swimming in contaminated water and accidentally swallowing water during swimming.

An important environmental concern about PCBs is their incorporation into the food chain. Benthic invertebrates feeding on the lake bottom consume PCBs and later pass them via the food chain to fish, birds, man, and other creatures. As PCBs pass through the food chain, concentrations accumulate and are transferred from one organism to another. As a result, the upper trophic levels are most prone to PCB accumulation (NTIS, 1979), and freshwater fish are the major source of PCBs in the diet of humans (Durfee et al., 1976). For instance, the concentration of PCBs in Lake Michigan averages approximately 0.008 pg/kg while PCB concentrations found in the lake trout are approximately 28 mg/kg (Metcalf et al., 1975). This concentration is over 3 million times that of the external

environment. It has been indicated that adults consuming 150 g per week of Coho salmon from Lake Michigan also are ingesting 15 mg/kg of PCBs, whereas in Japan, 80 g of fish contain the same quantity of PCBs (Hurkat, 1977). Studies of 70-79-year-old Japanese men revealed 5.1 mg/kg of PCBs in their fat. Japanese women of the same age bracket had 2.4 mg/kg PCBs in their fat (End et al., 1979). A 1972 survey by Yobs of 637 Americans from 18 states indicated that 26% had l-2 mg/kg PCBs in fat (Larson et al., 1979). In the same year, a report by the U.S. Department of Agriculture listed PCB presence in many common foods such as cheese (0.25 mg/kg), milk (2.27 mg/kg), and eggs (0.55 mg/kg) (Clayton and Clayton, 1994).

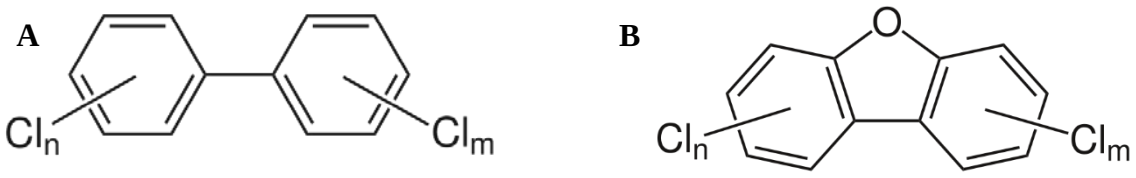

Figure 6. Chemical structures of PCBs (A) and PCDFs (B).

1.2.4.2 Toxicity

More recent outbreaks of poisoning in men and domestic animals from accidental food contamination with PCBs have resulted in the termination of commercial production of PCBs and regulation of their residues in food.

Rats that consumed food containing large amounts of PCBs for short periods of time had mild liver damage, and some died. Rats, mice, or monkeys that consumed smaller amounts of PCBs in food over several weeks or months developed various kinds of health effects, including anemia, acne-like skin conditions, and injuries in liver, stomach, and thyroid gland. Other effects caused by PCBs in animals include reductions in immune system function, behavioral alterations, and impaired reproduction. Some PCBs can mimic or block the action of hormones from the thyroid and other endocrine glands. Because hormones influence the normal functioning of many organs, some of the effects of PCBs may result from endocrine changes (ATSDR, 2000). Many studies have reported PCB effects on human health. Some of these studies have investigated people exposed in the workplace, and others have examined members of the general population. Some studies in workers suggest that exposure to PCBs may also cause irritation of the nose and lungs, gastrointestinal discomfort, changes in the blood and liver, and depression and fatigue (Craddock, 1982). Studies of workers also provide evidence that PCBs were associated with certain types of cancer in humans, such as cancer of the liver and biliary tract. Rats that ingested commercial PCB mixtures throughout their lives developed liver cancer. Based on the evidence for cancer in animals, the Department of Health and Human Services (DHHS) has stated that PCBs may reasonably be anticipated to be carcinogens. Furthermore, both EPA and IARC have determined that PCBs are probably carcinogen for humans (ATSDR, 2000).

On the other hand, another evaluation of PCB toxicity revealed that human health is not seriously jeopardized by PCB exposure. Direct evidence linking PCB exposures to

thyroid morbidity in humans is limited. PCBs have not been shown to be carcinogenic in humans, and only dermatitis, chloracne, rashes and other skin conditions have been conclusively linked to PCB exposure. These effects on the skin are well documented, but are not likely to result from exposures in the general population. These adverse effects are completely reversible and disappear if PCB exposure is terminated.

The exact effects of PCBs are difficult to ascertain because the mixtures often contain impurities. PCB mixtures have also been demonstrated to contain several other types of chlorinated compounds such as polychlorinated naphthalenes and polychlorinated dibenzofurans (PCDFs) (Figure 6B). The presence of these PCDFs in PCB mixtures may arise from the distillation process during purification (NTIS, 1979).

1.2.4.3 Mitigation of polychlorinated biphenyls exposure

There are several means by which PCBs can be eliminated from the environment. Hydrolysis can be used under extreme conditions, although PCBs are usually inert to this reaction (ICT, 1976). Chemical degradation is another method that can be used to eliminate PCBs (Brooks, 1982). The stability of PCBs is so great that environmental conditions are not likely to promote chemical reactions. However, under controlled conditions, oxidation, reduction, nitration, isomerization, and nucleophilic reactions can occur with PCBs (Hutzinger et al., 1974). For example, PCBs have been shown to be degraded to polyphenylene and sodium chloride by treatment with metallic sodium (George et al., 1988).

Photodegradation is another method for destruction of PCBs. Dechlorination of PCBs as well as the production of polymerized materials occur in hexane when exposed

to irradiation in sunlight. Chlorine can be replaced by hydrogen or hydroxyl groups, a condensation or rearrangement may occur, or even the production of polar products can result from such a reaction. Specifically, Aroclor 1254 can be degraded to hydroxylated and carboxylated species by irradiating it in the presence of hydroxylic solvent at pH 9. Very little photodegradation of PCBs occurs naturally because they are usually not readily accessible to sunlight.

Another natural process that can eliminate PCBs from the environment is biodegradation. A report indicates that natural soil processes will degrade PCBs (Brown et al., 1985). In this process, both position and degree of chlorination of PCBs play crucial roles in determining whether or not microbial degradation will occur. Bacteria can transform biphenyls of lower chlorination, but encounter difficulty with more highly chlorinated biphenyls (Onishi and Trench, 1981). Conversion of PCBs to chlorobenzoic acids is the main process of biodegradation. Not only do *Acinetobacter* bacteria follow this pathway, but so do other genera of bacteria such as *Alcaligenes, Arthrobacter, Achromobacter, Nocardia,* and *Pseudomona*s (Furukawa et al., 1983).

The final method for the destruction of PCBs is incineration. As PCBs are incinerated, hexachlorobenzene is formed. Both PCBs and hexachlorobenzene can be destroyed thermally at a temperature of 950°C (Lehman, 1965). Heating may cause PCBs to form different polymeric compounds incorporating oxygen (Zitko and Choi, 1971). Heating PCBs to 500-600ºC will cause the formation of degradative products such as chlorinated dibenzofurans (Buser et al., 1978).

Despite these methods, PCBs remain a persistent problem in the environment. Of the 60,420 kg of PCBs produced in the United States since 1930, more than half (60%) are still in service and 12% are mobile in the environment. Only 28% of these PCBs have been eliminated, 5% by incineration or chemical degradation and 23% by being placed in landfills (NTIS, 1979).

1.3 Clay research to reduce exposure in human populations

Simple, cost-effective and safe strategies to reduce human and animal exposures to toxin-contaminated food and water are warranted, especially in vulnerable populations.

Innovative strategies that significantly diminish toxin bioavailability and mitigate human and animal exposures from contaminated food, feed and water have been reported. Based on the extant scientific literature, some of these approaches are already in the stage of clinical intervention and translation. Studies describing materials that tightly adsorb aflatoxins onto internal and/or external surfaces interfering with toxin uptake and bioavailability have recently been reviewed (Kenseler et al., 2013; Miller et al., 2014; Phillips et al., 2019). Extensive studies with montmorillonite clay and dietary chlorophyllin in humans and animals indicate that they are approaching implementation, but still require further clinical evaluation in the field to delineate the effects of dose and time on efficacy and safety as well as acceptability (Phillips et al., 2002, Wild and Turner, 2002). Other aflatoxin sequestering materials that have limited evidence of efficacy will require preclinical trials in animals to confirm safety followed by clinical intervention trials in humans prior to implementation. Before full-scale implementation, all of these products should be rigorously evaluated *in vitro* and *in vivo,* and should meet the following

criteria: (1) favorable thermodynamic characteristics of toxin sorption, (2) tolerable levels of potential hazardous contaminants, (3) safety and efficacy in multiple animal species, and (4) safety and efficacy in long-term studies. Based on these criteria, certain calcium and sodium montmorillonite clays meet these requirements and have been used as the base material in our laboratory for the development of broad-acting clay-based sorbents for diverse toxins

The concept of eating dirt (clay) falls under the scientific term, geophagy, which is practiced by humans and animals alike. For centuries, people have used clays in food preparation for toxin removal, condiments or spices, or food during famine (Callahan, 2003). Other common clay consumption practices include their use as medication or during pregnancy with the latter being most common in cultures of sub-Saharan Africa (Callahan, 2003). The observation that populations at high risk for exposure commonly engaged in geophagy, as well as the success of zeolite, bentonite and spent bleaching clay from canola oil refining in reducing the effects of the T-2 and zearalenone mycotoxins in swine led to the investigation of the sorbent properties of montmorillonite clays. In the early 1980s, groundbreaking work in the Phillips laboratory demonstrated the efficacy of for aflatoxins (Phillips et al., 1987; 1988). Base on this previous work, isothermal analyses and molecular modelling techniques have been routinely employed to characterize and validate clay-based materials for the enterosorption of aflatoxins and other important mycotoxins.

Calcium montmorillonite falls under the phyllosilicate class. The functionality of this class of minerals is a result of the distinctive structural and chemical properties of the

silicate layers containing both tetrahedral and octahedral sheets. The tetrahedral sheets are composed of SiO_4 tetrahedra linked together with each sharing three O^{2-} ions with an adjacent tetrahedral. Together, this forms a plane of basal oxygens. The fourth O^{2-} of each tetrahedron is referred to as the apical oxygen and is free to bind to other elements. The octahedral sheet is comprised of two planes of OH^- groups that form a hexagonal close packing arrangement. In the case of CM, to counter the negative charge of this structure, Al^{3+} fills two out of every three spaces to produce a dioctahedral arrangement. With this structure, the apical oxygens from the tetrahedral layer coordinate with Al^{3+} to link the octahedral and tetrahedral layers in a 2:1 layer structure in which an octahedral layer is bound on either side by a tetrahedral layer. Frequently, cations in either the tetrahedral or octahedral layers are missing or have been replaced through an isomorphic substitution with another cation of lesser charge which results in a permanent negative surface charge. To counteract the negative charge, calcium montmorillonite clays predominantly attract Ca^{2+} into the region between the layers (i.e., the interlayer). This Ca^{2+} attraction into the interlayer allows for the space responsible for the high binding capacity of montmorillonite for toxins (Phillips et al., 2002; Schulze et al., 1989).

Montmorillonite has been used as an anticaking additive for animal feeds and was identified as an attractive mitigating agent due to its GRAS (Generally Recognized as Safe) classification. Based on multiple animal and human studies, montmorillonite clays have been confirmed to be safe for animal and human consumption, and effective for aflatoxin adsorption, with high binding capacity, affinity and enthalpy. Recent spinoffs from these studies have also resulted in the development of field-practical and cost-

effective sorbents for the mitigation of environmental chemicals. People and animals can be unintentionally exposed to mixtures of environmental chemicals and mycotoxins following natural and man-made disasters through contaminated water and food. To decrease potential human and animal exposures, we have developed clay-based enterosorbents that will decrease the bioavailability of complex chemical mixtures when included in the diet.

1.3.1 Sorbent amendments

To attract lipophilic chemicals, parent montmorillonite clays are amended with nature organic cations to increase the surface lipophilicity. L-carnitine (Figure 7A) is a quaternary ammonium compound which serves as an important enzyme cofactor for metabolism including the oxidation and transportation of fatty acids. It is essential for fatty acid translocation and muscle function (Brass and Hiatt, 1994; Bremer, 1997). Studies also showed carnitine supplement improves exercise performance. Choline (Figure 7B) is an essential water-soluble vitamin that is involved in amino acid metabolism and prevents deposition of fat in the liver. It is an essential nutrient for humans (National Academy of Sciences, 1998; Zeisel et al., 1991) and provides structure to cell membranes and facilitates transmembrane signaling as well as synthesis and release of acetylcholine (Buyukuysal et al., 1995; Zeisel and Blusztajn, 1994). It has been observed that there is a decline in plasma choline concentration in athletes after running a marathon (Conlay et al. 1992). The potential use of choline supplementation for improving physical performance has been reported (Hongu and Sachan, 2003). Deficiencies in L-carnitine and choline have been reported to be associated with liver disease, and cardiac and muscle metabolic

diseases. Since L-carnitine and choline are included in diets as essential nutrients and are considered to be safe, the potential for partial dissociation from clay surfaces would not be of concern.

With the permanent positive charge at the quaternary ammonium cation, both L-carnitine and choline can bind strongly to the negative surfaces of interlayers and at edge sites of montmorillonite clays. At 100% cation exchange capacity, amended sodium montmorillonite clays have been shown to be stable, heat resistant, and able to adsorb certain hydrophobic herbicides (i.e., terbuthylazine and diuron) (Celis et al., 2007). Previous studies by Jaynes and Zartman (2011) using amended low-charge sodium montmorillonite clays, showed increased binding for aflatoxin from aqueous corn flour. Also, the treatment of modified clays with organic cations has been applied successfully with water filtration and decontamination, metal and mineral adsorption, color bleaching, and removal of organics (Alther, 1995; Boyd et al., 1988; Dias Fiho and do Carmo, 2006; Gunawan et al., 2010; Liu and Zhang, 2007; Mortland et al., 1986; Wiles et al., 2005). The molecular mechanism for this effect apparently involves changing the nature of clay surfaces from hydrophilic to hydrophobic, which attracts organophilic chemicals to the active surfaces.

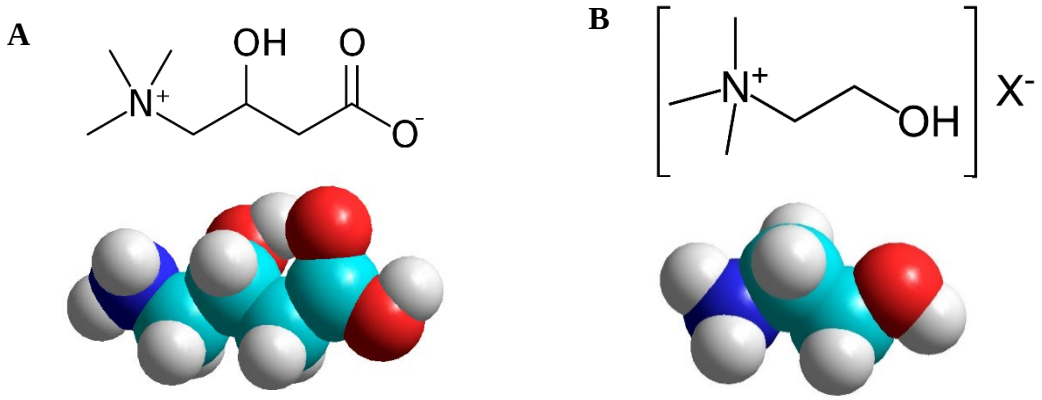

Figure 7. Chemical structures and molecular models of L-carnitine (A) and choline (B).

1.3.2 Acid processed sorbents

Exposure of people and animals to mixtures of chemicals is inevitable. However, strategies to minimize unintended exposures to contaminated drinking water and food during natural disasters and emergencies have not been developed. To address this problem, my research has focused on the development of novel, clay-based sorbents containing active surfaces that are broad-acting for a variety of important toxins, such as AfB_1, ZEN and diverse Superfund chemicals. Based on previous literature, the treatment of clays, such as montmorillonites, with high concentrations of acid results in the exchange of interlayer cations with protons from the acid, following the partial dissociation of octahedral and tetrahedral sheets in the clay structure. The final reaction product of acid processed montmorillonite (APM) clay is thought to be a mixture of delaminated parent clay layers containing chains of amorphous silica on the edges, amorphous silica, and cross-linked silica. The acid attack on clay minerals has additional effects on the mineralogical composition of the raw material and related properties; that is, organic

matter is leached out and feldspar can be partially attacked (or dissolved). This process causes an increase in surface area and surface acidity, introduces permanent mesoporosity and also removes metal ions from the crystal interlayer which partially delaminates the clay. During the strong acid treatment, a large increase in pore volume and an increase in larger pore size distribution were observed (Amari et al., 2018).

The pH of the final APM product is approximately 3, which is similar to acidic foods such as meat, cheese, and chocolate. In fact, the inclusion of acidic food additives can serve as a common taste enhancer and increase palatability and consumption. Also, acids, such as sulfuric acid, are permitted as food and feed additives to adjust pH. Thus, the inclusion of APMs at a low concentration in the diet of humans and animals for short-term treatments should be safe.

As a consequence of these structural changes, acid-treatment can extend the applications of these porous solids, i.e. the control of atmospheric pollution (Amari et al., 2010), bleaching (Akar and Uysal, 2010; Amari et al., 2018; Ullah et al., 2015), removal of plant pigments (i.e. chlorophyll) from oils and organic solutions (Guler and Tunc, 1992; Mokaya et al., 1994), and sequestering various organic and inorganic contaminants from water during decontamination and purification procedures (Erdogan and Sakizci, 2012; Kang and Xing, 2007; Resmi et al., 2012; Zhang et al., 2017). However, there are no reports suggesting that these types of materials (APMs) might be included in the diets of animals and humans for short-term treatment to decrease exposure to toxins from contaminated water and food.

1.4 Research objectives

Concerns about the quality and safety of our environment have evoked a growing awareness of the hazards associated with exposure to mycotoxins, PAHs, pesticides, organic solvents, plasticizers, and heavy metals. Many of these chemicals can have severe and long-lasting effects on human health. The scope of the problem is complicated by the risk of exposures to complex mixtures of hazardous chemicals in water and food following manmade and natural disasters, such as droughts, hurricanes and flooding. Although extensive research has focused on environmental remediation following disaster emergencies, strategies to minimize unintended exposures to contaminated drinking water and food during these disasters have not been developed. Based on our previous work developing materials that can mitigate aflatoxin outbreaks, we proposed that similar strategies can be used to decrease the risk of chemical exposures during disasters. Reducing exposures to complex chemical mixtures using broad-acting sorbents is not only a ground-breaking approach, but also field-practical and cost-effective. Sorbents developed in this study are derived from naturally occurring materials that are "generally recognized as safe" for short-term consumption and are predicted by biological screening and computational methods to be effective in reducing exposures and the toxicity of diverse contaminants and contaminant mixtures. The findings from this research are of direct relevance to first responders, vulnerable communities, and site remediation personnel exposed during environmental emergencies.

The major objective of this study was to develop broad-acting sorbents (based on montmorillonite clays) for the binding of chemicals and mixtures. Clay-based

enterosorbent therapy has been previously reported in both animal studies and human clinical trials, confirming the safety and efficacy of montmorillonite clay inclusions in diets. We have investigated the binding parameters of natural and amended sorbents using equilibrium isotherms to measure binding affinity, capacity, and enthalpy. We have also characterized the thermodynamics and fundamental mechanisms involved in the resulting chemical/surface interactions between these sorbent materials and chemicals. In addition, a Cnidarian model system (*Hydra vulgaris*) was used as a living organism to predict the toxicity and efficacy of sorbents at the whole animal level against reconstituted ("design") and "real-life" chemical mixtures. The optimal sorbents that were developed have shown high affinity, capacity, and enthalpy (heat of sorption) for complex mixtures of commonly occurring mycotoxins, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins/furans, high use industrial solvents, plasticizers, pesticides and heavy metals. We anticipate that the best broad-acting sorbents developed from this study will be included in diets of humans and animals as a protective measure to reduce toxin bioavailability and minimize unintended exposures to chemical contaminants during disasters and emergencies. Therefore, the principle goals of this research were to:

1) Screen and characterize prioritized test chemicals and sorbents for surface interactions, including affinities, capacities, and thermodynamics. We have selected 50 representative chemicals in the classes of mycotoxins, plasticizers, pesticides, metals, industrial solvents, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. Detection methods for individual chemicals have been developed and validated using HPLC, LC/MS/MS and UV/Visible scanning spectrometry.

Equilibrium isothermal analyses were conducted to investigate chemical/sorbent surface interactions. We validated binding by determining indices of chemisorption, specificity, affinity (K_d), capacity (Q_{max}), and enthalpy (ΔH) of adsorption from isothermal analyses. In addition, dosimetry predictions for sorbent inclusion were conducted to predict sorbent inclusion levels required to meet the regulatory level of each test chemical. We studied the correlation between chemical descriptors and properties (i.e., functional groups, logP, surface area, size, volume, etc.) and binding parameters. Finally, we confirmed *in vivo* the efficacy and safety of broad-acting sorbents for individual chemicals using a freshwater Cnidarian model (*Hydra vulgaris*) that is highly sensitive to environmental contaminants such as those found at Superfund sites.

2) Screen and characterize prioritized sorbent mixtures for safety and efficacy. The binding efficacy of each sorbent was investigated for 50 representative chemicals and correlated with chemical properties. To develop broad-acting mitigation therapies, sorbent mixtures that could bind common mixtures of chemicals were developed. The inclusion of each sorbent in the sorbent mixture was based on the safety, efficacy, and the minimum effective dose estimated from isotherms, hydra bioassays and dosimetry predictions. The binding ability of prioritized sorbent mixtures was tested *in vivo*.

3) Evaluate and confirm sorbent mixture efficacy with “designed” and “real-life” environmental mixtures using *in vivo* analysis. In this aim, we created “design mixtures” that were reconstituted to closely match pollutants found at contaminated sites based on our previous experience and EPA reports. We also evaluated different sets of “real-life”

chemical mixtures from water and sediment samples collected from Hurricane Harvey, and other contaminated sites from Montana and Washington State. Chemical mixtures are generally more toxic than a single chemical, thus require sorbents at higher inclusion levels, or sorbent mixtures for effectiveness. We selected sorbents and sorbent mixtures for the mitigation of environmental chemical mixtures based on binding efficacy and safety of individual sorbents shown by isothermal analyses, hydra bioassays and dosimetry predictions. The enterosorbent therapy developed from this project will be: 1) broad-acting, 2) capable of rapidly reducing human and animal exposures, 3) adaptable for a wide range of environmental chemical, mixtures and microbes.

2. DEVELOPMENT OF HIGH CAPACITY ENTEROSORBENTS FOR AFLATOXIN B1 AND OTHER HAZARDOUS CHEMICALS*

2.1 Introduction

Aflatoxins are secondary fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They are widespread and cause problems especially during extended periods of heat and drought. Fungal growth in food is favored by over 85% relative humidity and 25°C (Phillips et al., 1995). Among the 16 naturally occurring aflatoxins, aflatoxin B_1 (AfB_1) (Figure 8) is the most toxic and commonly detected in various food and feed products (Grant and Phillips, 1998). Symptoms caused by AfB_1 in animals and humans include growth stunting, weight loss, liver toxicity, immunosuppression and cancer (Murugesan et al., 2015).

Previously, our laboratory has conducted a series of *in vitro* studies, which have shown that Novasil Plus (NSP), a 2:1 layered calcium montmorillonite, is a unique sorbent that can tightly bind AfB_1. Its inclusion into feedstuffs and food has been reported to protect numerous animal species and reduce biomarkers of AfB_1 exposure in humans (Awuor et al., 2017; Phillips et al., 1995; Maki et al., 2016; Mitchell et al., 2014). The mechanism of this protection involves adsorption of AfB_1 onto active interlayer surfaces of NSP, resulting in reduced concentration of unbound toxin in the gastrointestinal tract

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and decreased bioavailability and toxicity of AfB_1 (Phillips, 1999).

Sodium montmorillonite (although similar in structure to NSP) is considered a less selective sorbent due to its high expansibility (swelling) in water and its potential for nonspecific binding. This study was aimed to improve clay-based sorbents for AfB_1 by modifying a calcium-rich montmorillonite (NSP) and a sodium-rich montmorillonite (Swy2) with L-carnitine and choline. L-carnitine is an important enzyme cofactor for metabolism including the oxidation and transportation of fatty acids (Chen et al., 2014). Choline is an essential water-soluble vitamin that is involved in amino acid metabolism (Rajaie and Esmaillzadeh, 2011). Deficiencies in L-carnitine and choline have been reported to be associated with liver disease, and cardiac and muscle metabolic diseases (Bonner et al., 1995; Rajaie and Esmaillzadeh, 2011; Yonei et al., 2008). Since L-carnitine and choline are included in diets as essential nutrients and are considered to be safe, the potential for partial dissociation from the clay would not be of concern. At 100% cation exchange capacity, amended sodium montmorillonite clays have been shown to be stable and heat resistant, and able to adsorb certain hydrophobic herbicides (i.e., terbuthylazine and diuron) (Celis et al., 2007). Previous studies by Jaynes and Zartman (2011), using amended low-charge sodium montmorillonite clays showed increased binding for aflatoxin from aqueous corn flour (Jaynes and Zartman, 2011). Also, the treatment of modified clays with organic cations has been applied successfully with water filtration and decontamination (Alther, 1995; Boyd et al., 1988; Mortlan et al., 1986; Wiles et al., 2005), metal and mineral adsorption (Dias Fiho and do Carmo, 2006), color bleaching (Gunawan et al., 2010; Liu and Zhang, 2007), and removal of organics (Gitipour et al., 2010), etc.

The molecular mechanism for this effect apparently involves changing the nature of clay surfaces from hydrophilic to hydrophobic, which attracts organophilic chemicals to the active surfaces.

Although parent NSP serves as one of the most specific and effective AfB_1 sorbents, isothermal analysis indicates that it binds 30% of the toxin at a concentration equal to 8 ppm. When included in the diet at 0.25% w/w, it decreases biomarkers of exposure in animals and humans by approximately 30-60% (Binghan et al., 2004; Harvey et al., 1989; Maki et al., 2016; Mitchell et al., 2014; Sarr et al., 1995). Based on earlier work with herbicides, it is postulated that L-carnitine and choline modified montmorillonites may sorb aflatoxins better than parent clays due to their hydrophobicity, thus increasing the binding capacity for toxin *in vitro* and *in vivo*. This study was designed to: 1) investigate the affinity and capacity of nutrient-amended montmorillonite surfaces for aflatoxins and, 2) predict their ability to prevent adverse effects of aflatoxin B_1 using the adult hydra assay.

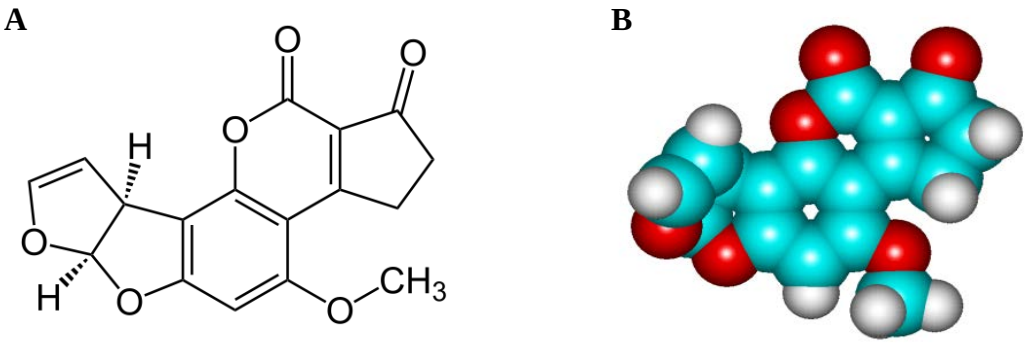

Figure 8. Structure of AfB_1 (A) and a molecular model (B) illustrating the spatial orientation and size of the functional groups.

2.2 Materials and methods

2.2.1 Reagents

High Pressure Liquid Chromatography (HPLC) grade methanol, acetonitrile reagents and pH buffers (4.0, 7.0 and 10.0) were purchased from VWR (Atlanta, GA). AfB_1, pepsin, L-carnitine and choline were purchased form Sigma Aldrich (Saint Louis, MO). NovaSil was obtained from Engelhard Corp (Cleveland, OH) and was sieved through 45 μm. Swy2 was a gift from the Source Clay Repository at Purdue University. Ultrapure deionized water (18.2 MΩ) was generated within the lab using an Elga™ automated filtration system (Woodridge, IL).

2.2.2 Synthesis of sorbents

Parent NSP and Swy2 sorbents were modified with the L-carnitine and choline at 100% cation exchange capacity (CEC = 97 cmol kg^{-1}). Calculated amounts of cations and 2 g of parent materials were added in 40 mL of 1 mM HNO_3. The suspensions were mixed and stirred for 24 h at ambient temperature, then centrifuged at 2000 g for 20 min and washed with 100 mL distilled water. This centrifugation-washing process was repeated three times. All samples were dried in the oven at 110℃ overnight before grinding and passing through a 125 μm sieve.

In order to confirm the importance of an intact interlayer and investigate cation and toxin binding sites, experiments with heat-collapsed sorbents were conducted. Collapsed sorbents were prepared by heating parent and amended sorbents at 200℃ for 30 min and 800℃ for 1 h to collapse the interlayer (Grant and Phillips, 1998).

2.2.3 Coefficient of linear expansibility in water

Sorbent samples were added to the 2 mL mark in graduated cylinders, then stirred with 15 mL of water. After 24 h following thorough equilibrium hydration and swelling, the final sorbent volume was determined. The ratio calculated from the beginning (2 mL) and final volume is indicative of hydration and expansion of the sample. A higher ratio indicates greater hydration and expansion of the sample.

2.2.4 In vitro isothermal adsorption

The AfB_1 stock solution was prepared by dissolving pure crystals into acetonitrile. A calculated amount of the stock solution was injected into pH 6.5 of distilled water to yield an 8 ppm (8 μg/mL) AfB_1 solution, which was confirmed by scanning and reading the absorption at 362 nm using a UV-visible spectrophotometer. The maximum AfB_1 concentration was set as 8 ppm (well below the solubility range of 11-33 ppm) so that precipitation of AfB_1 did not occur in the solutions (Meylan et al., 1996). Then 100 μg of sorbents were exposed to an increasing concentration gradient of AfB_1 solution: 0.4, 0.8, 1.6, 2.4, 3.2, 4, 4.8, 6, 6.4, 7.2 and 8 ppm. The concentration gradients of AfB_1 solutions were achieved by adding a calculated amount of 8 ppm solution along with a complementary volume of distilled water to sterile 17 x 100 mm polypropylene centrifuge tubes to make a total volume of 5 mL. The 100 μg sorbent inclusion was achieved by injecting 50 μL of 2 mg/mL clay suspension, which was mixed vigorously during the transferring to the sorbent/toxin mixture by an autopipetter. Besides testing samples, there were 3 controls consisting of 5 mL of distilled water, 5 mL of 8 ppm AfB_1 solution without sorbent and 5 mL of 100 μg sorbent in distilled water. The control and test groups were

capped and agitated at 1000 rpm for 2 h at ambient temperature using an electric shaker. All samples were then centrifuged at 2000 g for 20 min to separate the clay/AfB_1 complex from solution. The UV-visible spectrophotometer was used to measure the absorption of AfB_1 in the supernatant from samples and controls.

2.2.5 Data calculations and curve fitting

The UV-visible absorption data were used to calculate the concentration of AfB_1 left in solution (c) by Beer's law (x-axis). The amount adsorbed for each data point (y-axis) was calculated from the concentration difference between test and control groups. More specifically, the y-axis is the amount of toxin bound by sorbents (in mol/kg). It is calculated by the difference in moles of free toxin in the test solution versus control groups and is then divided by the mass of the clays included.

Beer's law $\qquad\qquad$ Absorbance = ε L c

ε is the molar extinction coefficient (ε for AfB_1 = 21,865 $cm^{-1}mol^{-1}$), L is the path length of the cell holder = 1 cm, dependent on the cuvette.

These data were then plotted using Table-Curve 2D and a computer program that was developed with Microsoft Excel to derive values for the variable parameters. The best fit for the data was a Langmuir model, which was used to plot equilibrium isotherms from triplicate analysis. The isotherm equation was entered as user-defined functions:

Langmuir model (LM) $\qquad\qquad q = \text{Qmax}\left(\frac{KdCw}{1+KdCw}\right)$

q = AfB_1 adsorbed (mol/kg), Q_{max} = maximum capacity (mol/kg), K_d = distribution constant, C_w = equilibrium concentration of AfB_1.

The plot will normally display a break in the curve. The value on the x-axis where the curve breaks is an estimate of K_d^{-1}. The value on the y-axis where the curve breaks is an estimate of Q_{max}. The Q_{max} is taken from the fit of LM to the adsorption data.

The definition of K_d is derived by solving for K_d from the Langmuir equation giving

$$Kd = \frac{\mathrm{q}}{(\mathrm{Qmax} - \mathrm{q})\mathrm{Cw}}$$

2.2.6 AfB_1 adsorption in a simulated stomach model

The gastrointestinal model previously reported was slightly modified to focus on the stomach in these studies (Beak et al., 2006; Rodriguez et al., 1999; Taylor and Phillips, 2010). Based on this model, an aqueous solution (pH 2) with 2 mg/mL pepsin was prepared in a water-jacketed beaker and maintained at 37°C. The concentration gradients of AfB_1 solutions were achieved by adding a calculated amount of 8 ppm solution along with the aqueous pepsin solution. Then 100 μg of sorbents were exposed to an increasing gradient of AfB_1 solution. The samples were capped and agitated at 1000 rpm for 2 h at 37°C. Samples were centrifuged at 2000 g for 20 min to separate the clay/AfB_1 complex. The absorption of AfB_1 was measured with a UV-visible spectrophotometer and the adsorption isotherms were plotted as described above.

2.2.7 Hydra assay

Hydra vulgaris were obtained from Environment Canada (Montreal, Qc) and maintained at 18°C. The hydra classification method (Wilby et al., 1990) was used with modification to rate morphology of the adult hydra as an indicator of solution toxicity. The illustration of this classification is indicated in Figure 9. In this assay, the scoring of

hydra morphology is objective and repeatable as indicated in previous literature. The assay included monitoring times at shorter intervals during the first two days (0, 4, 20, and 28 h) and 24 h intervals for the last three days (44, 68, and 92 h). Solutions were not changed during testing. The hydra morphological response was scored and recorded after exposure to AfB_1 with and without sorbent treatment. Mature and non-budding hydra in similar sizes were chosen for testing in order to minimize differences between samples. Controls for this experiment included hydra media consisting of 18.2 MΩ water, 4 mg/L EDTA, 115 mg/L N-tris[Hydroxymethyl]methyl-2-aminoethanesulfonic acid, and 147 mg/L $CaCl_2$ adjusted to pH 6.9-7.0. Sorbent inclusion percentage was chosen based on previous studies (Phillips et al., 2008; Marroquin-Cardona et al., 2011). Toxin treatment groups included 20 ppm AfB_1 in hydra media based on the minimum effective dose (MEC). MEC is the lowest dose that caused 100% mortality in 92 h. All test solutions were capped and prepared by shaking at 1000 rpm for 2 h and centrifugation at 2000 g for 20 min prior to toxin exposure of hydra in the Pyrex dishes (Phillips et al., 2008). For each sample, three hydra were included into 4 mL of test media and kept at 18°C. The score or average toxicity rating was determined by calculating the average score for morphological changes for a certain group at a specific time point.

2.2.8 Molecular models

The molecular model for montmorillonite was drawn in ISIS Draw 2.0 and then imported into HyperChem 8.0. The aflatoxin, carnitine and choline structures were energy-minimized using the semiempirical quantum mechanical AM1 method. The model was constructed using the unit cell coordinates of muscovite (Richardson and Richardson,

1982). These coordinates were then converted to orthogonal coordinates in an Excel spreadsheet that was constructed from a public domain C program. The unit cells were replicated in three-dimensional space by applying the symmetry operations for a C2/c space group (Donnay, 1952). The d_{001} spacing of the model was then set to the corresponding dimensions of the exchanged montmorillonite (21 Å) based on the report of Greenland and Quirk (1960). Aflatoxin, carnitine and choline were inserted into the interlayer and on the external surface (Slade and Emerson, 1978) to illustrate the proposed sites of aflatoxin adsorption.

2.2.9 Statistical analysis

A one-way t-test was used to calculate statistical significance. Each experiment was independently triplicated to derive an average and standard deviation. In the t-test, the average COLE ratio from COLE experiments, Q_{max} from equilibrium isothermal analyses and toxicity scores from the hydra assay were included to calculate D = control-test groups and D^2.

The t-value and DF (degrees of freedom) were compared in a p-value table to determine the statistical significance. Results were considered significant at $p \leq 0.05$.

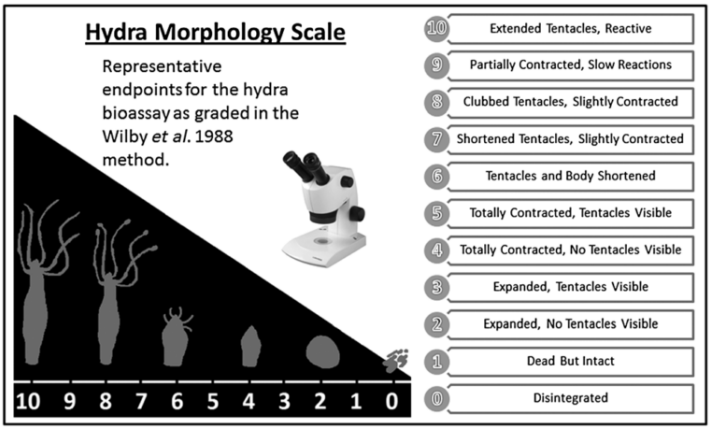

Figure 9. Hydra morphology scale by the Wilby method, 1988: The scale is graded from 0-10, where 10 represents a normal living hydra and 0 represents a disintegrated hydra. The physiologic conditions of hydra were assessed with a dissecting microscope.

2.3 Results

2.3.1 Coefficient of linear expansibility in water

The COLE ratio indicates the expansibility of sorbents in water. COLE = expansion volume of clay/original volume of clay. The higher the ratio, the more expansion and hydration of the sample. The accuracy of this experiment was confirmed by the COLE values of NSP and Swy2 clays, which predicted calcium-rich and sodium-rich montmorillonite, respectively. The COLE ratios for exchanged clays with L-carnitine and choline values were 1.1 and 1.2, showing very limited swelling compared to their parent sorbents as shown in Figure 10.

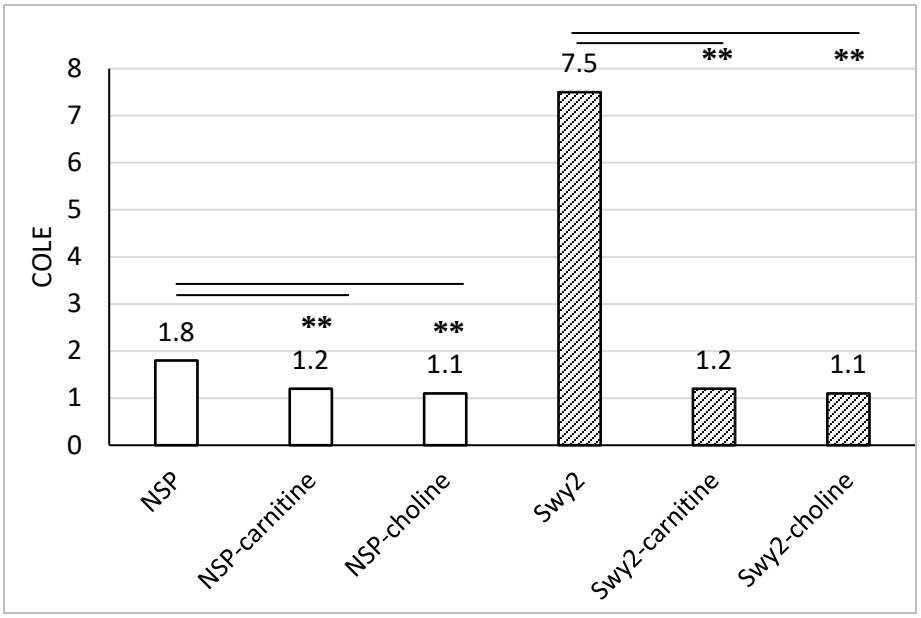

Figure 10. Coefficient of linear expansibility for sorbents in water. The COLE value for Swy2 indicated significant hydration and expansibility, whereas COLE values for other sorbents displayed very limited hydration energy and expansibility. Statistical significance is denoted by $^{*}p < 0.05$ and $^{**}p < 0.01$.

2.3.2 Isothermal adsorption and analyses for AfB_1

Equilibrium isotherms were generated by Table-Curve 2D and a computer program developed in our laboratory using Microsoft Excel. This program was used to derive affinities (K_d), capacities (Q_{max} in mol AfB_1 bound kg^{-1} sorbent) and the enthalpy of sorption for toxin-surface interactions. Based on r^2 values and randomness of the residuals, the best fit for the data was a Langmuir model, which was used to plot equilibrium isotherms from triplicate analyses. Each point represents the values calculated for AfB_1 bound to clay (mol/kg) and AfB_1 left in solution (mol/L) for the corresponding 11 dilutions. Isotherms were performed at ambient temperature and light.

Figure 11 shows the AfB_1 isothermal plot on Swy2 and NSP modified clays. For all isotherms, the r^2 values (or coefficients of determination) were above 0.8, indicating that the raw data strongly fit the Langmuir model and that AfB_1 binds tightly onto clay surfaces and does not dissociate easily. The Q_{max} derived for Swy2-carnintine and Swy2-choline are significantly increased from that of parent Swy2 sorbent. The Langmuir plots on NSP-modified clays also indicates an increase in binding capacity for aflatoxin. Carnitine and choline improved the binding of aflatoxin by NSP and Swy2, although the effect was slightly better for Swy2. The K_d values for parent and amended clays were similar. In another isothermal plot simulating the stomach model with the addition of pepsin at pH 2, the AfB_1 adsorption on NSP and NSP-carnitine both decreased dramatically compared to the Q_{max} derived from the control treatment. Interestingly, adsorption of AfB_1 decreased by 73% on parent NSP (Q_{max} = 0.08), whereas adsorption only decreased by 21% on NSP-carnitine (Q_{max} = 0.26) as shown in Figure 12. After the interlayers were collapsed from heating at 800℃, the AfB_1 adsorption was dramatically decreased (Figure 13). Collapsed NSP and NSP-carnitine both decreased in their capacity to bind aflatoxin with Q_{max} values equal to 0.03 and 0.004, respectively.

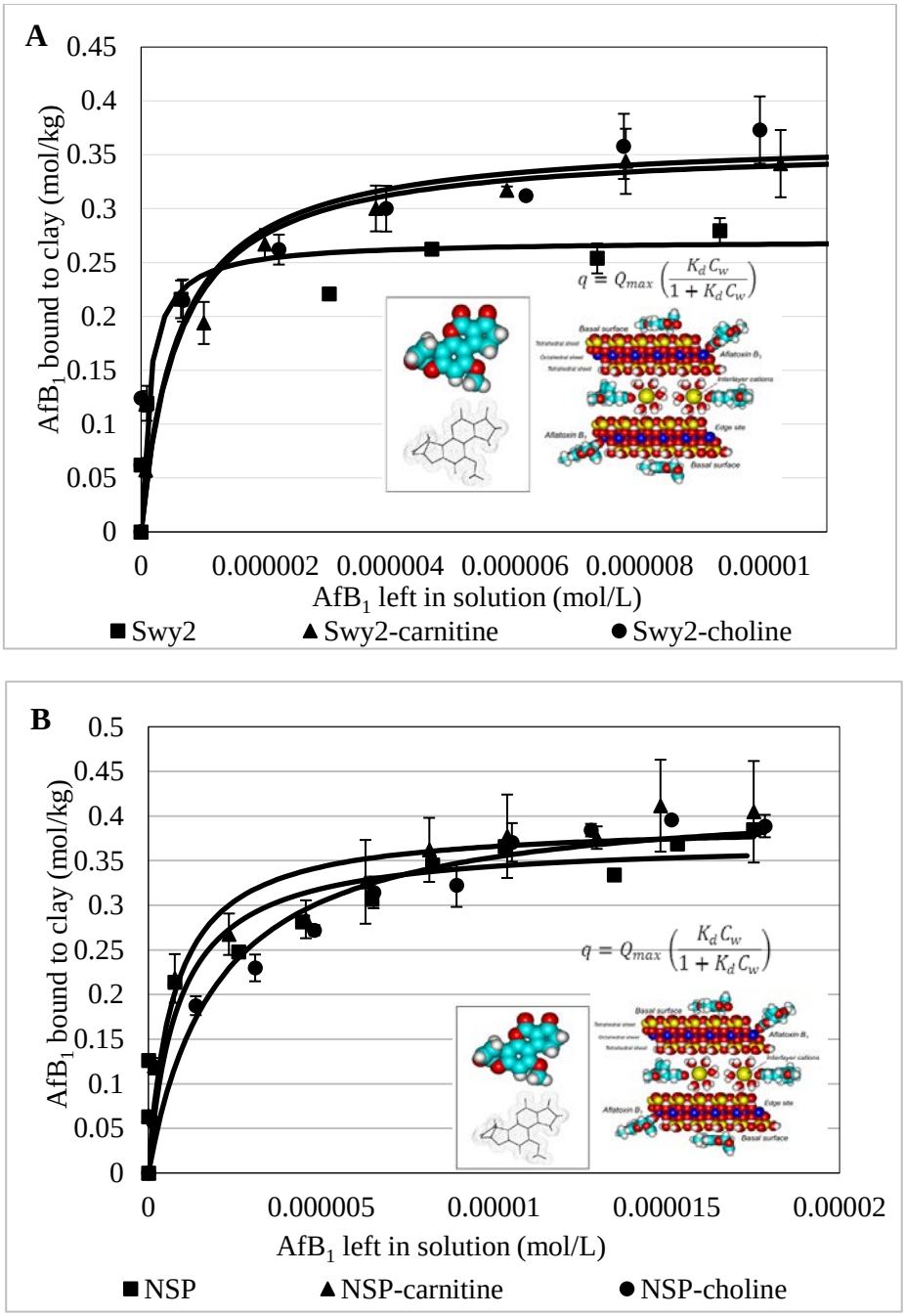

Figure 11. Langmuir plots of AfB_1 on Swy2 (A) and NSP (B) showing the observed and predicted Q_{max} values at pH 6.5. The Q_{max} values indicated tight binding for all three sorbents. Swy2: Q_{max} = 0.27; K_d = 7E6; Swy2-carnitine: Q_{max} = 0.36; K_d =2E6; Swy2-choline: Q_{max} = 0.367; K_d = 2E6. NSP: Q_{max} = 0.37; K_d = 1E6; NSP-carnitine: Q_{max} = 0.39; K_d = 1.4E6; NSP-choline: Q_{max} = 0.42; K_d = 5E5.

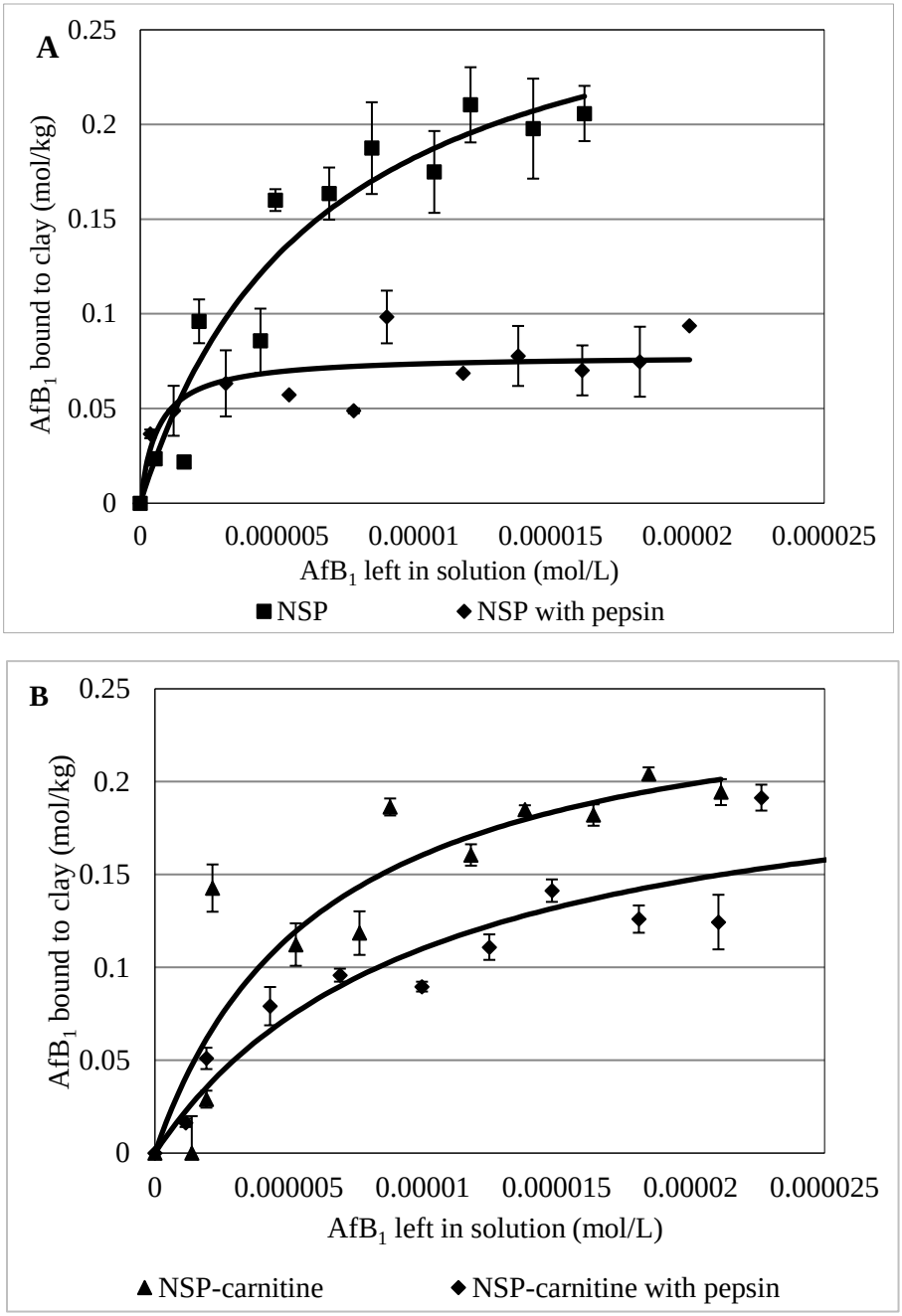

Figure 12. Langmuir plots of AfB_1 in a simulated stomach model of NSP (A) and NSP-carnitine (B) at pH 2. The Q_{max} values indicated tight binding for NSP and NSP-carnitine sorbents in the stomach model. NSP: Q_{max} = 0.3; K_d = 1E5; NSP-carnitine: Q_{max} = 0.26; K_d = 1E5; NSP with pepsin: Q_{max} = 0.08; K_d = 1.5E6; NSP-carnitine with pepsin: Q_{max} = 0.22; K_d = 1E5.

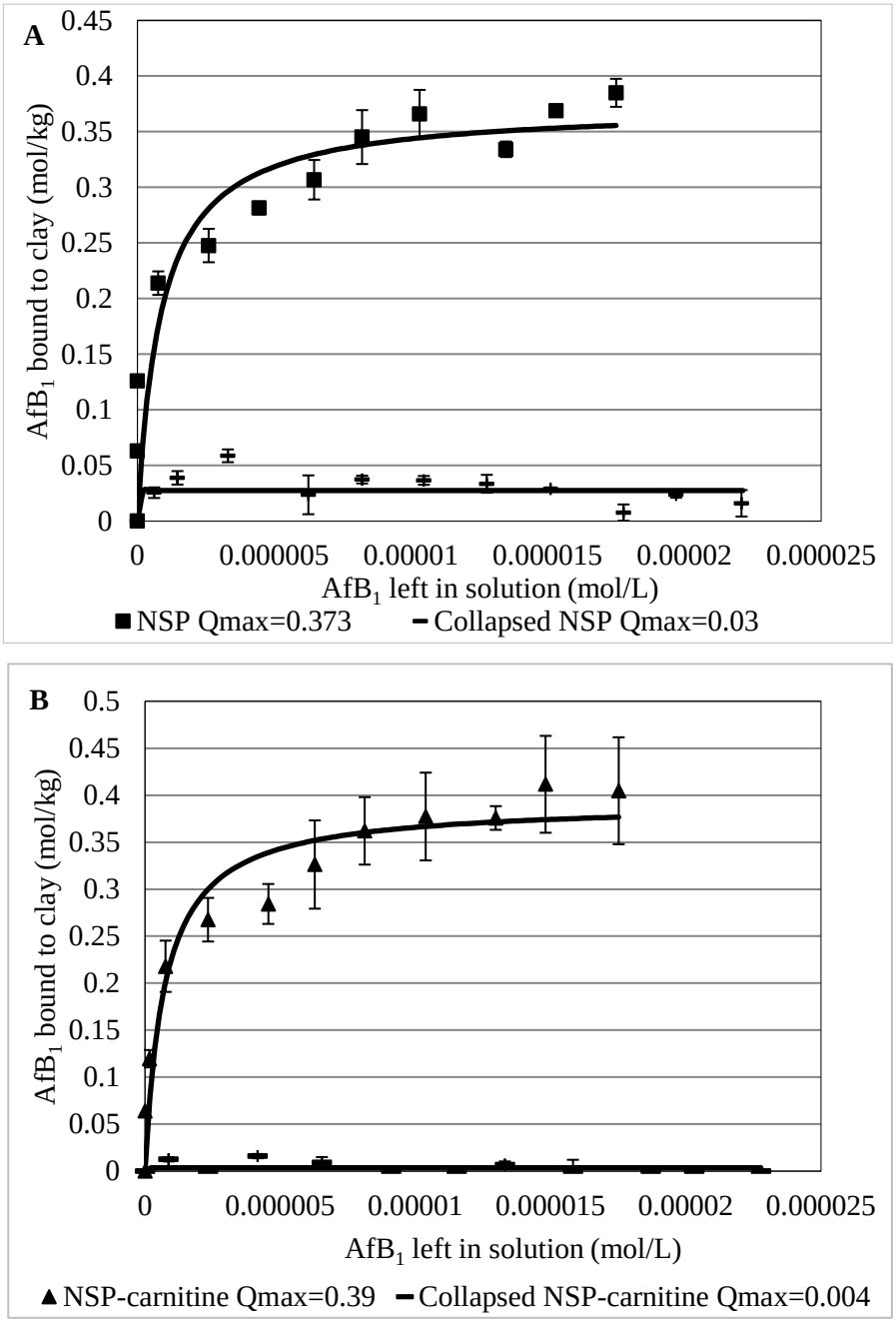

Figure 13. Langmuir plots of AfB_1 on collapsed NSP (A) and collapsed NSP-carnitine (B) at pH 6.5. The Q_{max} values for NSP and NSP-carnitine indicated tight binding, whereas the Q_{max} values for collapsed NSP and collapsed NSP-carnitine indicated very limited binding.

2.3.3 Hydra assay

The minimal effective concentration for AfB_1 has been established earlier at 20 ppm, which results in 100% hydra mortality in 92 h. Amended sorbents at very low inclusion rates of 0.005% rendered a significant protection for hydra against AfB_1, resulting in morphologic ratings not different from the hydra media control group. Whereas at the same inclusion levels for unmodified NSP and especially Swy2, the scores are similar to the AfB_1 control group, showing no protection derived from these parent sorbents at 0.005% (Figure 14). Based on the isothermal and hydra results above, the estimated molecular models for aflatoxin, L-carnitine and choline on surfaces of montmorillonites are shown in Figure 15.

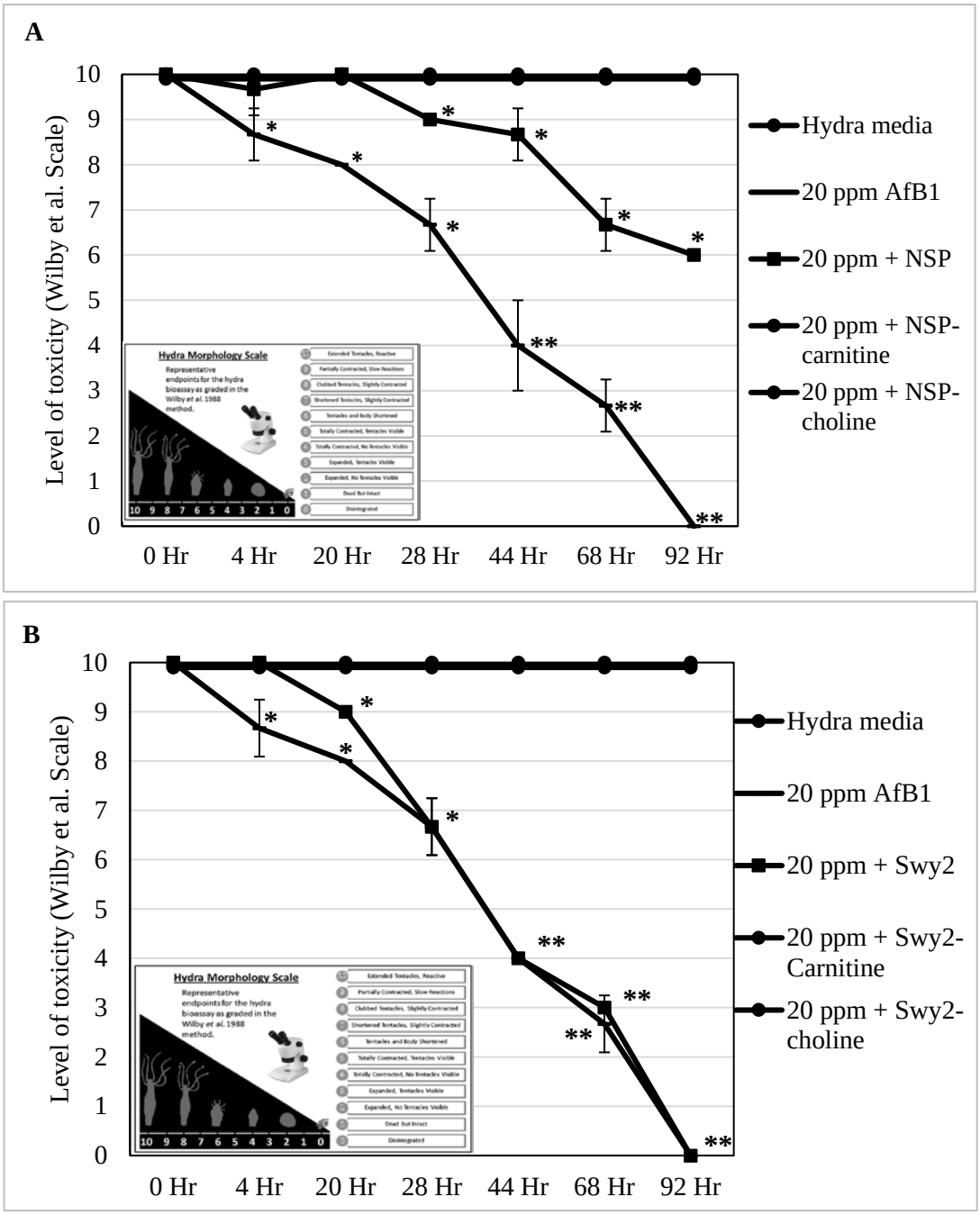

Figure 14. Hydra toxicity and protection by parent and amended NSP (A) and Swy2 (B) at 0.005% inclusion. All four amended sorbents were able to protect the hydra at all time points from the toxic effects of AfB_1. Hydra media and toxin controls were included in each figure for comparison.

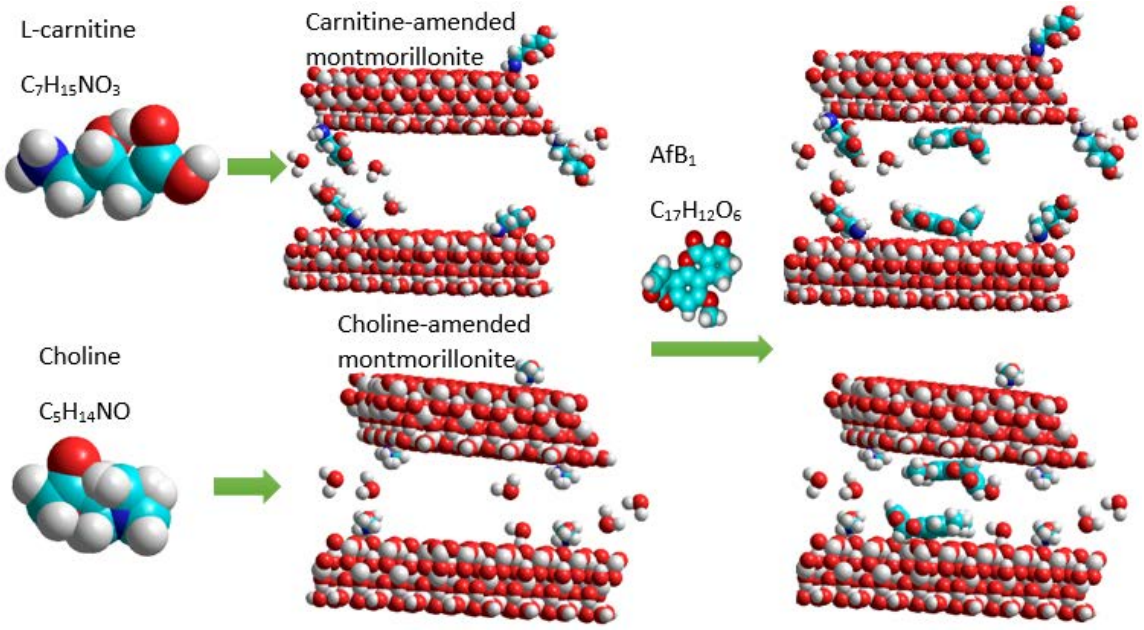

Figure 15. Computational model of AfB_1 binding onto the surfaces of carnitine and choline amended montmorillonites.

2.4 Discussion

Based on our previous *in vitro* and *in vivo* studies, calcium montmorillonite was the most effective aflatoxin enterosorbent. The interlayers of sodium-rich montmorillonite clays are more accessible than calcium montmorillonite, but less selective due to their expansibility in water.

Swy2 is a sodium montmorillonite with high expansibility as indicated by its COLE ratio of 7.5. Modified Swy2 sorbent displayed a larger decrease in expansibility in water, compared to NSP. This is probably due to cation exchange and decreased hydration energy in the amended sorbents with carnitine and choline. The smaller sodium ion is more readily hydrated and dissociated than the divalent calcium ion in NSP, explaining

the difference in swelling activity between the 2 clays. This restriction of expansibility in water and stabilization of clays by L-carnitine and choline may serve to make these amended clays more effective as toxin enterosorbents by limiting the access of other potentially competing chemicals (such as pepsin) into interlayer channels. For example, calcium montmorillonite has been reported to sorb aflatoxins and fumonisins preferentially with very little interaction with nutrients, whereas sodium montmorillonite (with higher expansibility) has been shown to bind a wider variety of toxins and nutrients such as xanthophylls (Gray et al., 1998).

NSP (calcium-rich montmorillonite) and Swy2 (sodium-rich montmorillonite) amended with L-carnitine and choline can change the mineral surface polarity and water occupation in the interlayer, and facilitate the incorporation of aflatoxin molecules in the interlayer of the montmorillonite. As AfB_1 is only slightly soluble in water based on its high K_{ow} (octanol/water partition coefficient) with an estimated solubility range of 11-33 ppm, this change in polarity facilitates the sorption of aflatoxins to amended clay surfaces. For example, amendment of Swy2 with choline and L-carnitine resulted in a higher capacity for aflatoxin sorption versus the parent Swy2. A higher capacity was also observed with amended NSP.

Although the expansibility of Swy2 in water is larger than NSP, its binding capacity was typically lower, as shown in Figure 11. Isothermal analysis indicated that sodium montmorillonite amended with L-carnitine and choline markedly increased the binding capacity for aflatoxin. In fact, Swy2-carnitine and Swy2-choline showed the potential to be applied as efficient AfB_1 sorbents comparable to NSP since the Q_{max} of

amended Swy2 and NSP are similar. The fact that K_ds are similar in parent and amended Swy2 indicates that the binding affinity is not affected by the addition of modifiers. It is possible that L-carnitine and choline replaced hydrated inorganic cations (mainly sodium and calcium) and balanced platelet surface charge, exposing hydrophobic surfaces that favor AfB_1 adsorption. This modification by L-carnitine and choline provides a novel approach to stabilize swelling sorbents like Swy2, and increase the AfB_1 binding capacity similar to NSP.

In Figure 11, NSP amended with L-carnitine and choline both improved aflatoxin binding based on a slightly higher binding capacity compared to the parent NSP. In comparison of isothermal results, amending with organic nutrients does not improve binding efficiency of NSP as much as Swy2, suggesting that L-carnitine and choline work best with swelling sorbents. However, both NSP and Swy2 amended with L-carnitine and choline were able to increase their binding capacity and the percentage of bound AfB_1 versus the parent sorbents. Importantly, the increase in binding capacity of NSP predicts its effectiveness *in vivo* (based on the hydra assay).

Pepsin as one of the major enzymes in the stomach was included in the 11 treatment dilutions at pH 2 to simulate a stomach model. Adsorption results in Figure 12 suggest that with pepsin at pH 2, aflatoxin binding by both parent and amended sorbents decreased due to competition at similar sites. The fact that AfB_1 decreased more with parent NSP than with NSP-carnitine indicates that L-carnitine amended clay favors AfB_1 binding by blocking the sequestration of pepsin and possibly other large proteins, and will be more effective as an aflatoxin enterosorbent when included in animal feeds.

After heating at 800°C, the interlayers of NSP and NSP-carnitine were dehydroxylated and collapsed. Figure 13 shows that binding capacities of collapsed NSP and collapsed NSP-carnitine are significantly reduced. This dramatic decrease of AfB_1 suggests (indirectly) that most of the AfB_1 binds within the interlayer of these clays and only minor amounts bind on the edges and basal surfaces. Since the binding capacity of collapsed NSP-carnitine is less than collapsed NSP, this indicates that L-carnitine is also bound on sites that were not bound by AfB_1. Based on isothermal results from heat-collapsed sorbents, we suspect that the addition of L-carnitine and choline (at acidic pH) neutralize interlayer surface charges, occupy polar sites, and create hydrophobic surfaces. More specifically, the interlayer surfaces of parent montmorillonite clays are negatively charged, which can be neutralized by the positive charges from the organic modifiers. This results in a reduction of polar charge which produces more hydrophobic surfaces that facilitate the binding of aflatoxin.

The protective roles of parent and amended clays were identified using the adult hydra assay. As indicated in the results, amended clays at a rate of inclusion as low as 0.005% resulted in significant protection of hydra against AfB_1, whereas at the same inclusion levels for unmodified NSP and Swy2, no protection was shown. Both the hydra assay and isotherm binding capacities derived for NSP and Swy2 indicated that parent NSP had better binding of aflatoxin and more protection of hydra than Swy2. Because this work was designed to improve the binding capabilities of clay-based sorbents, we only included low levels of clays (0.005%) in the hydra assay, which was considerably less than the common inclusion level in animal feed (0.2%). As a result, our modified clays

increased the binding capacity for aflatoxin and improved the protection of hydra. Unprocessed NSP clay has been shown to be an effective aflatoxin sorbent, but only at higher inclusion levels.

In summary, sorbent amendment with L-carnitine and choline results in effective protection of adult hydra against AfB_1. This finding *in vivo* is also consistent with our *in vitro* isothermal results. The molecular models of modified montmorillonite clays amended with L-carnitine, and choline are shown in Figure 15.With the permanent positive charge from the quaternary ammonium cation, both L-carnitine and choline can bind strongly to the negative surfaces of interlayers and at edge sites of montmorillonite clays.

Taken together, modifying NSP and Swy2 with carnitine and choline reduced the swelling of montmorillonites in water and increased their binding capacity and efficacy for aflatoxins. The increased binding from isothermal analyses (*in vitro*) was consistent with *in vivo* protection of adult hydra against AfB_1, suggesting that clay minerals (especially swelling montmorillonites) modified with L-carnitine and choline may be effective as enterosorbent therapy for aflatoxins. Our work significantly broadens the mycotoxin binding application of modified clays from a variety of previous studies. Janes and Zartman (2011) amended sodium montmorillonite instead of calcium montmorillonite and their results indicated that the amendment of low charge montmorillonite improved aflatoxin binding from contaminated corn flour solutions. Even in this complex matrix, amended montmorillonite clay was able to extract and bind aflatoxin. Our study extended this work using calcium and sodium clays with isothermal analyses in simulated stomach

conditions, computational chemistry to delineate potential mechanisms, and protection from aflatoxin in a living organism. Both of these studies support the conclusion that nutrient modifiers improve aflatoxin binding under different conditions. Further studies are warranted to determine the potential for dissociation of the modifiers (nutrients) and their potential interactions in GI tract as well as their safety and efficacy in animals and humans. Based on previous findings, we also postulate that these amended clays can be applied to environmental chemicals such as pesticides and PAHs with similar hydrophobicity. The screening of environmental chemicals is ongoing in our laboratory. Our ultimate goal is to develop broad-acting enterosorbents, which can be used to mitigate exposures to mixtures of hazardous toxins in food and feed following outbreaks, natural disasters, spills and emergencies.

3. DEVELOPMENT OF BROAD-ACTING CLAYS FOR THE TIGHT ADSORPTION OF BENZO[A]PYRENE AND ALDICARB*

3.1 Introduction

Natural and man-made disasters can significantly mobilize soil and sediment, exposing humans and animals to contaminated drinking water and food. A major challenge associated with these disasters and emergencies is the protection of: 1) vulnerable communities and neighborhoods, 2) first responders, and 3) those involved in management and cleanup of contaminated sites. We anticipate that the inclusion of broad-acting enterosorbents in diets will be a protective measure to minimize unintended exposures and the bioavailability of hazardous chemical contaminants.

Multiple classes of chemicals have been prioritized by the Agency for Toxic Substances and Disease Registry (ATSDR) as important chemicals, including various polycyclic aromatic hydrocarbons (PAHs), pesticides, metals, organic solvents, plasticizers, etc. In this study, benzo[a]pyrene (BaP), an acutely toxic and carcinogenic PAH, and aldicarb, a widely used and highly toxic pesticide, were selected for study based on their toxicities and extensive distribution. BaP is a well-known environmental pollutant and a human and animal carcinogen. BaP is commonly found in contaminated water and sediment after natural disasters. The Environmental Protection Agency (EPA) reported

*Reprinted with permission from "Development of broad-acting clays for the tight adsorption of benzo [a] pyrene and aldicarb" by Wang, M., Hearon, S.E., Johnson, N.M., Phillips, T.D., 2019. *Applied Clay Science*, 168, 196-202, Copyright 2019 by Elsevier.

that BaP was detected at high contamination levels that exceed EPA's excess lifetime cancer risk range after Hurricane Katrina in 2005 and Hurricane Harvey in 2017 (apnews.com; epa.gov). Besides natural disasters, significant amounts of BaP were found in the dust and smoke after the World Trade Center disaster in downtown New York City (Xu et al., 2014). Aldicarb is an acutely toxic insecticide, acaricide, and nematicide that belongs to the carbamate class. The toxicity of carbamate insecticides, as well as organophosphorus compounds, is due to the inhibition of the enzyme acetylcholinesterase (Bertrand and Bertrand, 1991). Exposure to aldicarb can stimulate lipid peroxidation and paralyze the respiratory system (Yarsan et al., 1999). Aldicarb is one of the major pesticides found in water and sediment samples in the US and Canada, including groundwater and drinking water in New York and Wisconsin. Importantly, 50% of the concentration in New York groundwater was above the state standard of 7 ppb; 0.9% of samples contained aldicarb at concentrations above 100 ppb (Jones and Marquardt, 1987). Activated carbon contains pores and high specific surface area that provide surfaces for adsorption of toxins. Because of its sorptive properties for a wide range of chemicals, carbon is widely used in air and water purification, medicine, sewage treatment, etc. Previous studies have shown that activated carbon is the most efficient adsorbent for BaP and aldicarb (Zimmerman et al., 2004; Ayranci and Hoda, 2005; Brandli et al., 2008). However, during the production of activated carbon, incomplete combustion results in the formation of PAHs and other hazardous organic contaminants, making carbon's safety for human and animal consumption questionable (Mohammad-Khah and Ansari, 2009).

To develop safer alternatives to activated carbon, we selected a montmorillonite clay that has been previously shown to be safe for human and animal consumption (Awuor et al., 2017). We modified this montmorillonite clay with natural nutrients including L-carnitine and choline. Carnitine is an important enzyme cofactor for metabolism including the oxidation and transportation of fatty acids (Chen et al., 2014). Choline is an essential water-soluble vitamin that is involved in amino acid metabolism (Rajaie and Esmaillzadeh, 2011). Since carnitine and choline are included in diets as essential nutrients and are considered safe, the potential for partial dissociation from the adsorbent would not be of concern. At 100% cation exchange capacity, amended montmorillonites have been shown to be stable in solution, heat resistant, and able to adsorb certain hydrophobic herbicides and mycotoxins (i.e., terbuthylazine, diuron and aflatoxin) (Celis et al., 2007; Wang et al., 2017).

Besides amended montmorillonites, another natural product was investigated for its binding efficacy for BaP. Talc is a hydrated magnesium silicate that is widely used in baby powder, cornstarch, edible oils, plastics, lubricants, etc. Epidemiologic studies have suggested an association of ovarian cancer and perineal exposure to talc, but the correlation remains weak (Harlow et al., 1992; Wong et al., 1999; Berge et al, 2017). Talc is highly hydrophobic once water is adsorbed onto talc basal surfaces (Rotenberg et al., 2011; Tarasevich and Aksenenko, 2014), hence it is possible that talc has a high affinity for hydrophobic chemicals.

This study was designed to investigate and compare the binding of BaP and aldicarb with nutrient-amended montmorillonites, talc and activated carbon, and to predict efficacy in vivo using the adult hydra assay.

3.2 Materials and methods

3.2.1 Reagents

Reagents for high pressure liquid chromatography (HPLC), including methanol, acetonitrile and pH buffers (4.0, 7.0 and 10.0) were purchased from VWR (Atlanta, GA). BaP, aldicarb, ammonium acetate, magnesium chloride powder, L-carnitine and choline were purchased from Sigma Aldrich (Saint Louis, MO). Parent montmorillonite clay was obtained from Engelhard Corp (Cleveland, OH) and was sieved through 45 μm for testing. Talc (magnesium silicate monohydrate) and nicotinamide adenine dinucleotide phosphate (reduced tetrasodium salt) (NADPH) were purchased from Alfa Aesar (Ward Hill, MA). Ultrapure deionized water (18.2 MΩ) was generated within the lab using an Elga™ automated filtration system (Woodridge, IL). Rat (Sprague Dawley) S9 liver microsomes were purchased from GIBCO, Thermo Fisher Scientific (Waltham, MA). Dimethyl sulfoxide (DMSO) was purchased from Fisher Bioreagents (Fair Lawn, NJ).

3.2.2 Synthesis of sorbents

Parent montmorillonites were modified with L-carnitine and choline at 100% cation exchange capacity based on the method described previously by Wang, et al. (2017). Collapsed adsorbents were prepared by heating the amended adsorbents at 200℃ for 30 min and 800℃ for 1 h to collapse the interlayer completely (Grant and Phillips, 1998).

3.2.3 In vitro isothermal adsorption

The toxin stock solutions were individually prepared by dissolving pure crystals into acetonitrile to yield 10 ppm (µg/mL) BaP and 5 ppm aldicarb solutions. The maximum stock concentrations were set based on chemical solubility in the mobile phase to prevent the occurrence of precipitation. Then 0.002% w/w of adsorbents were added to toxin solutions with an increasing gradient. The concentration gradients of toxin solutions were achieved by adding a calculated amount of toxin stock solution along with a complementary volume of mobile phase in 1.5 mL centrifuge tubes to make a total volume of 1 mL. The 0.002% adsorbent inclusion was achieved by injecting 20 µL of 1 mg/mL clay suspension, which was mixed vigorously during transfer to the adsorbent/toxin mixture. Additionally, we tested 3 controls consisting of 1 mL of mobile phase, 1 mL of toxin solution without adsorbent and 1 mL of 0.002% adsorbent in mobile phase. The control and test groups were agitated at 1000 rpm for 2 h at ambient temperature (24°C) and 37°C for thermodynamic experiments. All samples were then centrifuged at 2000 g for 20 min to separate the clay/toxin complex from solution.

HPLC was used to measure the amount of free BaP in the supernatant (Rotenberg et al., 2011). Chromatography was conducted on a Waters HPLC equipped with a 717 plus autosampler, model 1525 binary pumps, a multi wavelength fluorescence detector and a Phenomenex® luna 5u C18 column (250 x 4.6 mm). Chemical separation was achieved by a mobile phase of 90% acetonitrile and 10% water at 1.0 mL/min flow rate and an injection volume of 100 µL. The fluorescent detector was set with excitation at 264 nm

and emission at 412 nm. The detection limit for BaP was 32 ppt. Breeze software was used to control the HPLC system and collect the data.

Alidicarb concentrations were analyzed using a Waters Acquity® ultra performance LC/MS/MS equipped with an Acquity® BEH C18 column (2.1 x 50 mm). The column temperature was kept at 35°C. A gradient elution using 10 mM NH4OAc in water (elute A) and 10 mM NH4OAc in methanol (elute B) was carried out (elute B, 10%-90% linear gradient for 8 min) at a flow rate at 0.6 mL/min. Sample volumes of 5 μL were used for each analysis. The mass spectrometer was performed with an electrospray ionization (ESI) interface and operated in a positive ion mode. The detection limit was 33.3 ppb for aldicarb. The spray voltage was maintained at 5 kV. Nitrogen gas was used as the collision gas and curtain gas, and argon gas was used as the nebulizer gas and heater gas. The source temperature was kept at 500°C. The mass spectrometer was operated under multiple reaction monitoring (MRM) mode and the monitored precursor and product ions were m/z 208.2 to 116.1. The unit mass resolution was used for ion mass analyzers. The EPI scan rate was 1000 amu/s, and the scan range was 106 to 396 amu. Empower analyst software was used to control the LC/MS/MS system and acquire the data.

3.2.4 Data calculations and curve fitting

Samples were prepared in triplicate and quantified using standard calibration curves. Therefore, the toxin concentration in solution (x-axis) detected by HPLC and LC/MS/MS was calculated from peak area at the toxin retention time. The amount adsorbed for each data point (y-axis) was calculated from the concentration difference

between test and control groups. These data were then plotted using Table-Curve 2D and a computer program that was developed with Microsoft Excel to derive values for the variable parameters. The best fit for the data was a Langmuir model, which was used to plot equilibrium isotherms from triplicate analysis. The isotherm equation was entered as user-defined functions:

Langmuir model (LM) $$q = \mathrm{Qmax}\left(\frac{KdCw}{1+KdCw}\right)$$

q = toxin adsorbed (mol/kg), Q_{max} = maximum capacity (mol/kg), K_d = distribution constant, C_w = toxin equilibrium concentration

The plot will normally display a break in the curve. The value on the x-axis where the curve breaks is an estimate of K_d^{-1}. The value on the y-axis where the curve breaks is an estimate of Q_{max}. The Q_{max} is taken from the fit of the Langmuir model to the adsorption data. The definition of K_d is derived by solving the Langmuir equation below:

$$Kd = \frac{\mathrm{q}}{(\mathrm{Qmax} - \mathrm{q})\mathrm{Cw}}$$

The enthalpy (ΔH) is a thermodynamic parameter indicating total heat released or absorbed during a reaction. It is calculated from the Van't Hoff equation by comparing individual Kd values at different temperatures (24°C and 37°C):

Van't Hoff equation $$\Delta\mathrm{H} = \frac{-\mathrm{R}\ln\left(\frac{\mathrm{Kd_2}}{\mathrm{Kd_1}}\right)}{\left(\frac{1}{\mathrm{T_2}}\right)-\left(\frac{1}{\mathrm{T_1}}\right)}$$

R (ideal gas constant) = 8.314 /Jmol/K, T (absolute temperature) = 273 + t (°C)

3.2.5 Hydra assay with a metabolism activation package

Hydra vulgaris were obtained from Environment Canada (Montreal, Qc) and maintained at 18°C. The hydra classification method (Wilby et al., 1990) was used with

modification to rate morphology of the adult hydra as an indicator of solution toxicity. In this assay, the scoring of hydra morphology is objective and repeatable based on previous work (Brown et al., 2014). Toxin treatment groups included 3 ppm BaP with a metabolism activation package (MAP) in 1% DMSO or 1 ppm aldicarb in hydra media based on the minimum effective dose that caused 100% hydra mortality in 92 h. The preparation of the MAP and the assay procedure was modified based on previous literature (Newman et al., 1990; Ottinger et al., 1999). MAP was standardized and consisted of 2.4 μg/mL mice hepatic microsomal cytochrome P450, 225 μM NADPH and 25 μM $MgCl_2$. All test solutions were capped and shook at 1000 rpm for 2 h with centrifugation at 2000 g for 20 min prior to exposure of hydra in the Pyrex dishes. For each sample, three hydra were included into 4 mL of test media and kept at 18°C. The score or average toxicity rating was determined by calculating the average score of morphological changes for a certain group at a specific time point.

3.2.6 Molecular Models

The molecular model for montmorillonite was drawn in ISIS Draw 2.0 and then imported into HyperChem 8.0. The BaP, aldicarb, carnitine and choline structures were energy-minimized using the semiempirical quantum mechanical AM1 method. The model was constructed using the unit cell coordinates of muscovite (Richardson and Richardson, 1982). These coordinates were then converted to orthogonal coordinates in an Excel spreadsheet that was constructed from a public domain C program. The unit cells were replicated in three-dimensional space by applying the symmetry operations for a C2/c space group (Donnay, 1952). The d001 spacing of the model was then set to the

corresponding dimensions of the exchanged montmorillonite (21 Å) based on the report of Greenland and Quirk (Greenland and Quirk, 1960). BaP, aldicarb, carnitine and choline were inserted into the interlayer and on the external surface (Slade et al., 1978) to illustrate the sites of toxin adsorption.

3.2.7 Statistical analysis

A two-way t-test was used to calculate statistical significance. Each experiment was independently triplicated to derive an average and standard deviation. In the t-test, K_d from equilibrium isothermal analyses and enthalpy analyses, Q_{max} from collapsed equilibrium isothermal analyses and toxicity scores from the hydra assay were included to calculate D = control-test groups and D^2. Then the t-value was calculated using the following equation (N = 3):

$$t = \frac{(\sum D)/N}{\sqrt{\frac{\sum D^2 - (\sum D)^2/N}{(N-1)N}}}$$

The t-value and degrees of freedom were compared in a p-value table to determine the statistical significance. Results were considered significant at $p \leq 0.05$.

3.3 Results

3.3.1 Isothermal adsorption and analyses

Equilibrium isotherms were generated by Table-Curve 2D and a computer program developed in our laboratory using Microsoft Excel to derive affinities (Kd), capacities (Q_{max} in mol of toxin bound/kg of adsorbent) and the enthalpy (ΔH in kJ/mol) of adsorption for toxin-surface interactions. Based on r^2 values and randomness of the residuals, the *best* fit for the data was a Langmuir model, which was used to plot

equilibrium isotherms from triplicate analyses. Each point represents the values calculated for toxin bound to clay (mol/kg) and free toxin in solution (mol/L) for the corresponding 5 dilutions.

Figure 16 shows that the isothermal plot of BaP on parent montmorillonite had a Freundlich trend, indicating a partitioning activity of BaP toxin onto clay surfaces. The r^2 values (> 0.8) for activated carbon, amended montmorillonites and talc indicate they fit the Langmuir model, and the curved shapes indicate that BaP binding was saturable and tight onto these clay surfaces and was not easily dissociated. This is aligned with the significant increase ($p \leq 0.01$) of the K_d values of amended clays, activated carbon and talc when compared to that of parent montmorillonite. The Q_{max} for carnitine and choline amended montmorillonites was a result of saturable and tight binding of BaP to the surfaces of these amended adsorbents, and was similar to the Q_{max} of activated carbon. Talc shows the highest Q_{max} and K_d values for BaP adsorption and these were considerably higher than that of activated carbon.

The results from Figure 17 show isothermal adsorption of BaP on carnitine and choline amended adsorbents at 24°C and 37°C. After applying individual K_d values at different temperatures into the Van't Hoff equation, the calculated enthalpies were: $\Delta H_{Mont\text{-}carnitine}$ = -42 kJ/mol; $\Delta H_{Mont\text{-}choline}$ = -46 kJ/mol. The high enthalpies suggest that the reaction involves chemisorption rather than physisorption, and the tight binding facilitates reduced bioavailability and toxicity. Based on our extensive earlier work with aflatoxin binding to montmorillonites, this type of data predicts that these new adsorbents will decrease bioavailability and toxicity of the toxins *in vivo*.

After heating at 800°C, the interlayers of amended clays were dehydroxylated and collapsed. Figure 18 shows that there was no statistical difference between the binding capacities of BaP on collapsed amended adsorbents versus intact adsorbents. This result suggests that the primary binding sites for BaP were the more organophilic basal surfaces and edge sites, which were not affected during the heat. Since many toxins bind at interlayer regions rather than basal surfaces and edge sites, we postulated that the amended clays are able to bind BaP and other toxins with limited interference due to the different binding mechanisms and sites.

The adsorption isotherm for aldicarb shows that amendment of montmorillonites with carnitine and choline improved from a Freundlich model (displayed by the parent adsorbent) to a Langmuir model with high binding capacities in Figure 19. This is confirmed by statistical analysis indicating a significant increase of K_d values with the amended montmorillonites. Following dehydroxylation and collapse of the amended clays (Figure 20), the adsorption activity changes from a Langmuir model to a Freundlich model. Significant differences were shown when comparing the K_d values of the collapsed clays. A summary table of adsorption parameters for BaP and aldicarb onto surfaces of amended montmorillonites is shown in Table 1. The molecular models representing BaP and aldicarb adsorption onto basal and interlayer surfaces of carnitine-amended montmorillonites are shown in Figure 21.

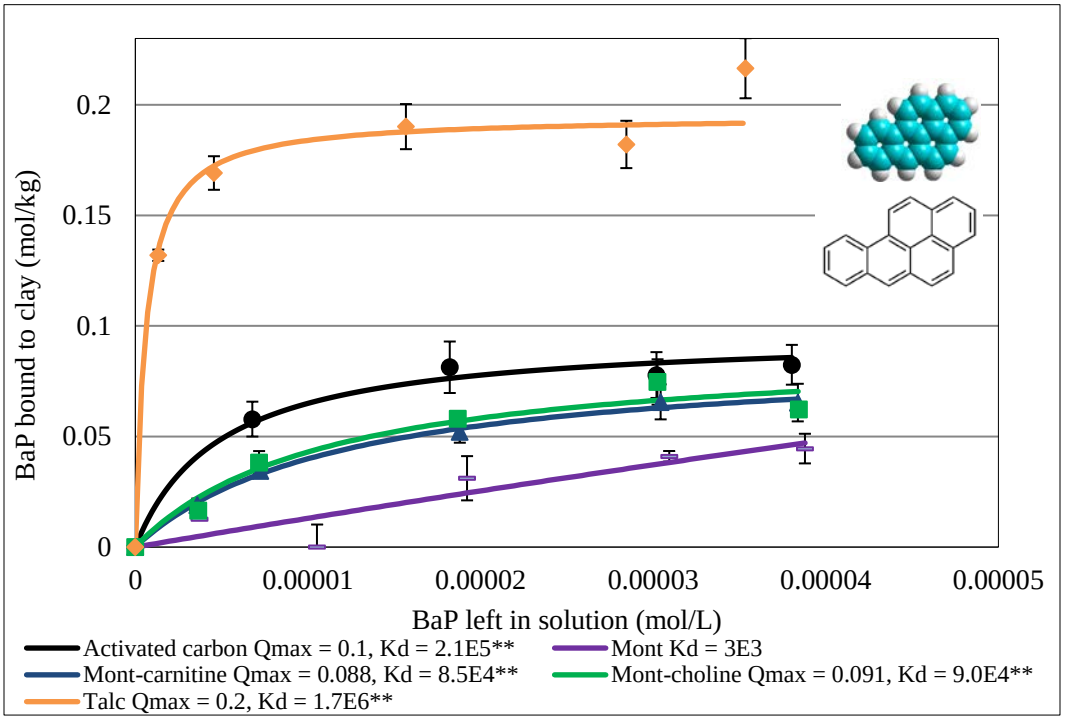

Figure 16. Isothermal plots of BaP on activated carbon, talc, parent clay and amended montmorillonites showing Q_{max} and K_d values ($^{**}p \leq 0.01$).

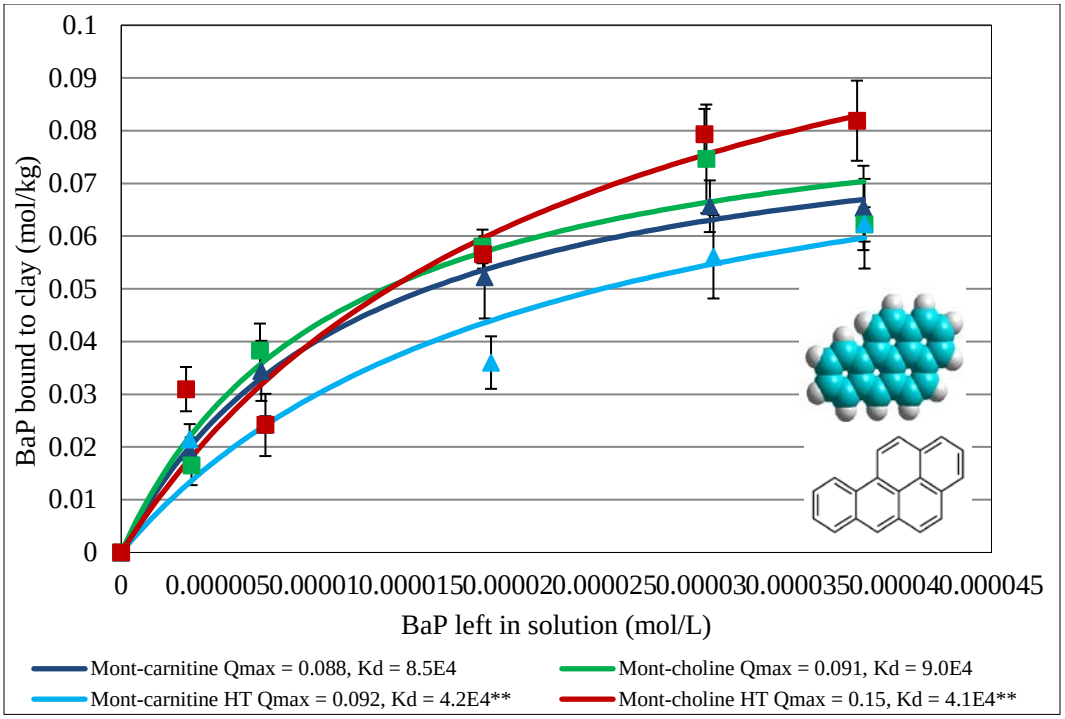

Figure 17. Isothermal plots of BaP on carnitine and choline amended montmorillonites at ambient and high temperatures (HT) ($^{**}p \leq 0.01$).

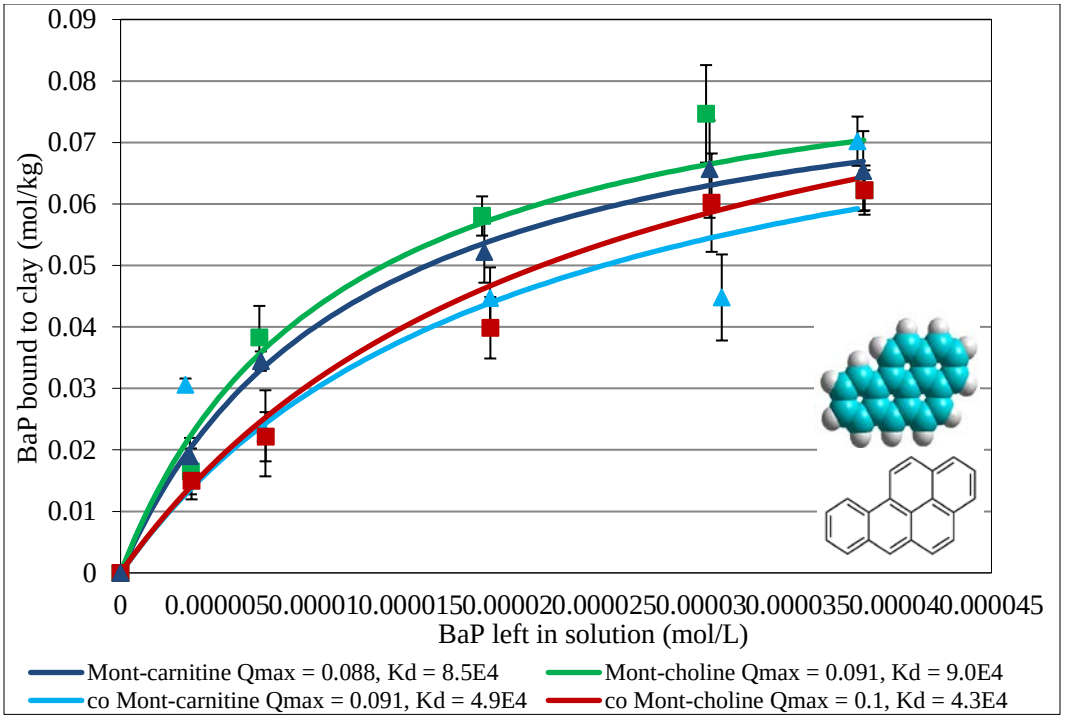

Figure 18. Isothermal plots of BaP on collapsed (co) carnitine and choline amended montmorillonites. No statistical difference was shown when comparing the Q_{max} of collapsed clay to the intact amended montmorillonite clays.

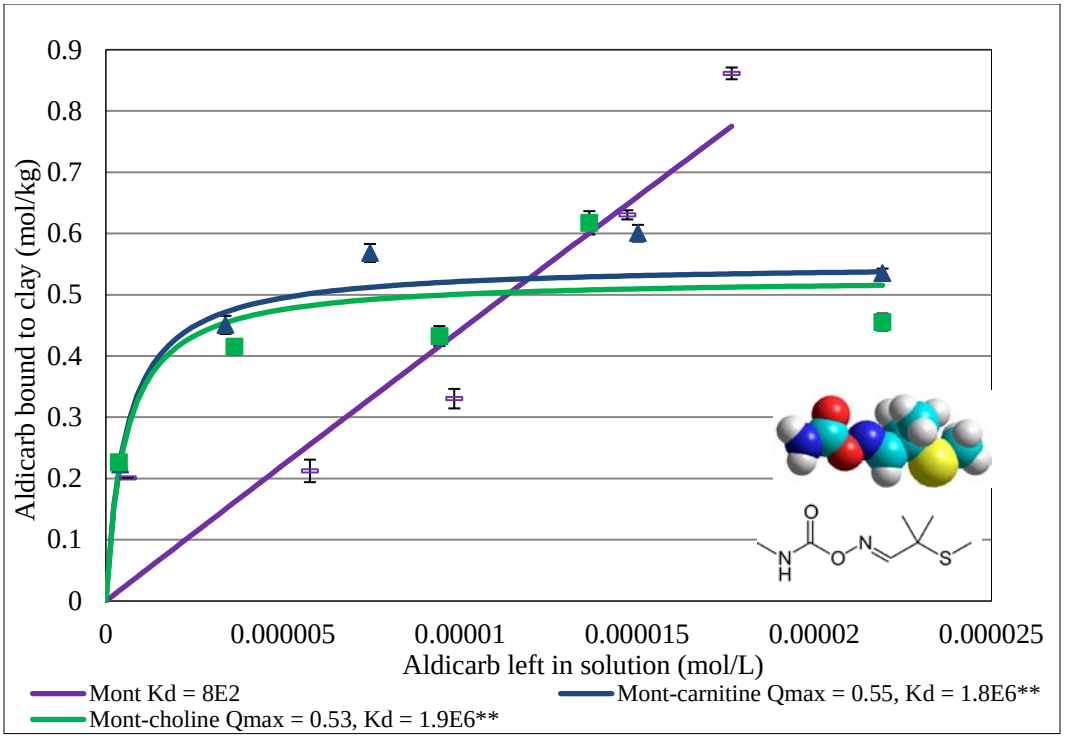

Figure 19. Isothermal plots of aldicarb on parent and amended montmorillonites showing Q_{max} and K_d values ($^{**}p \leq 0.01$).

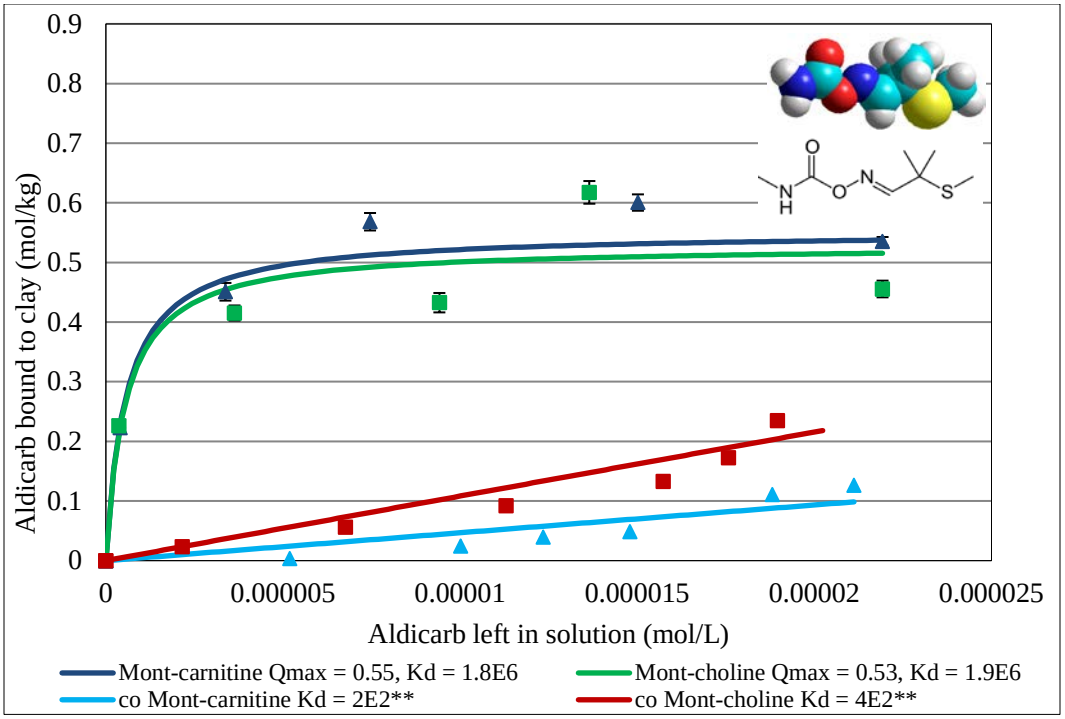

Figure 20. Isothermal plots of aldicarb on collapsed (co) carnitine and choline amended montmorillonites. Statistical differences were shown when comparing the K_d values of collapsed clay to the intact amended montmorillonite clays ($^{**}p \leq 0.01$).

Table 1. Summary table of binding parameters for amended montmorillonites

	BaP		Aldicarb	
	Q_{max}	K_d	Q_{max}	K_d
Mont-carnitine	0.088	8.5E4**	0.55	1.8E6**
Mont-choline	0.091	9.0E4**	0.53	1.9E6**
Mont-carnitine HT	0.092	4.2E4	N/A	N/A
Mont-choline HT	0.15	4.1E4	N/A	N/A
co Mont-carnitine	0.091	4.9E4**	Freundlich	2E2**
co Mont-choline	0.1	4.3E4**	Freundlich	4E2**

(** $p \leq 0.01$)

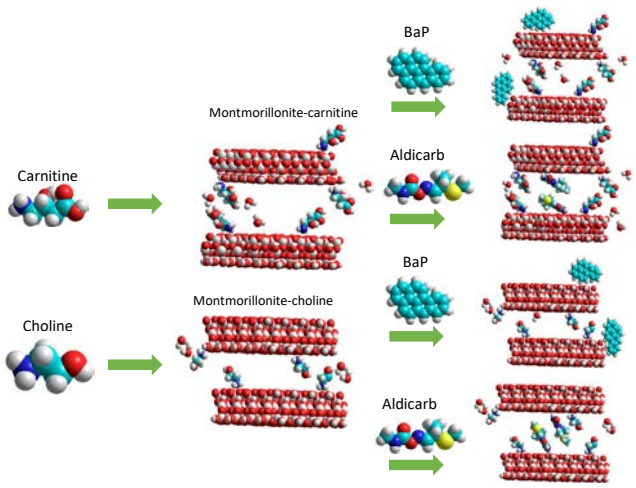

Figure 21. Computational models of BaP and aldicarb binding onto the surfaces of carnitine amended montmorillonites.

3.3.2 Hydra bioassay

Hydra media (with MAP and 1% DMSO) was not toxic to hydra. The toxin concentrations included in the hydra bioassay were set at 3 ppm BaP (with MAP) and 1 ppm aldicarb, resulting in 100% hydra mortality in 92 h. Amended adsorbents with carnitine and choline, and talc at low inclusion rates of 0.2% demonstrated obvious protection of hydra against BaP. At the same inclusion level for activated carbon, the morphologic rating trend was similar to the BaP control group, suggesting slight protection derived from this adsorbent. Carnitine and choline amended adsorbents were able to significantly protect against aldicarb toxicity at even lower inclusion rates of 0.1%, resulting in morphologic ratings not different from the hydra media control group (Figure 22).

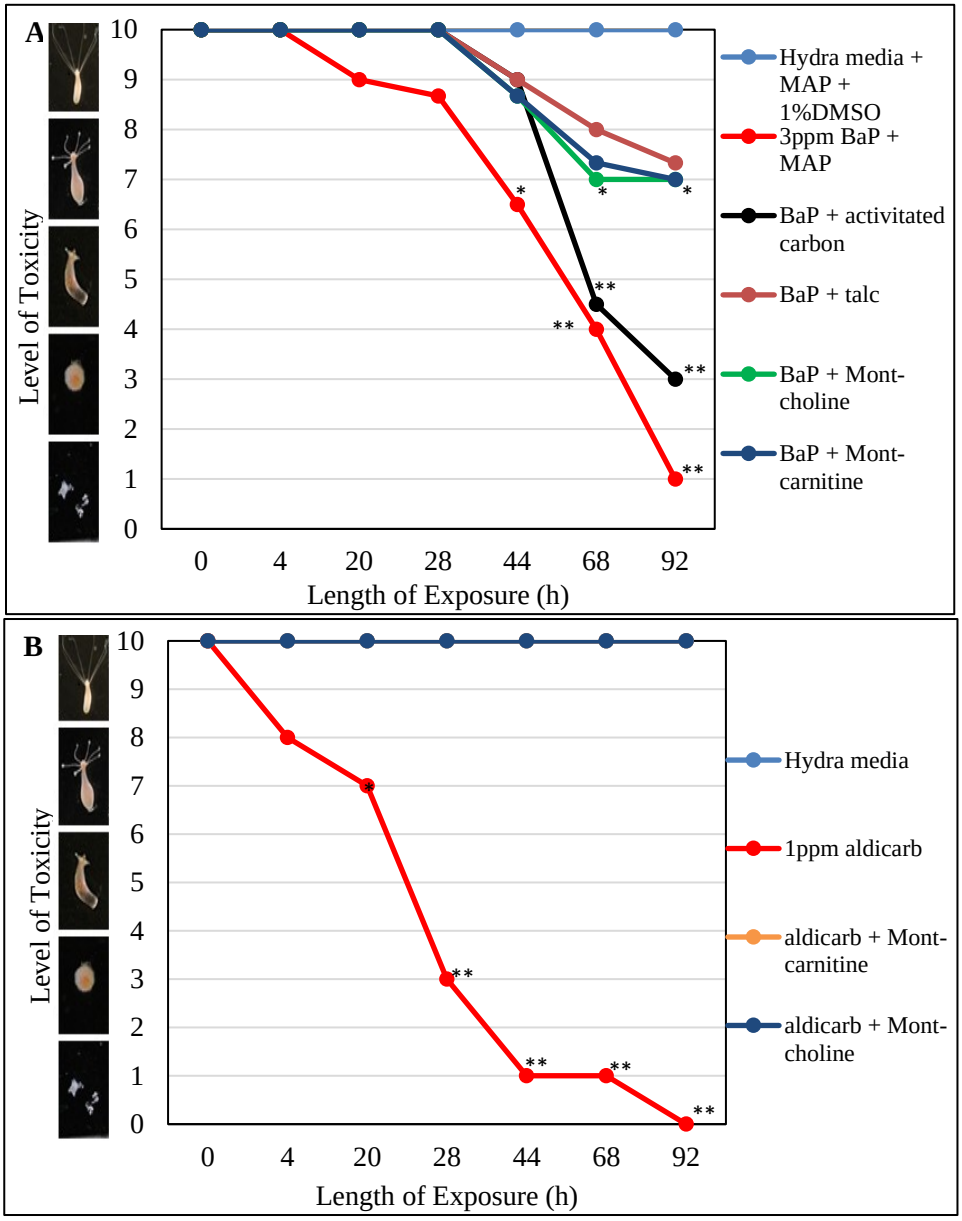

Figure 22. Hydra toxicity and protection by sorbent materials (at 0.2% inclusion) against BaP (A) and 0.1% inclusion against aldicarb (B). Both amended sorbents were able to protect the hydra at all time points from the toxic effects of BaP and aldicarb. Hydra media and toxin controls were included in each figure for comparison (* $p \leq 0.05$, ** $p \leq 0.01$).

3.4 Discussion

Parent base calcium montmorillonite clay has been shown to be safe for consumption without adverse effects based on previous clinical studies in humans and animals (Elmore et al., 2014; Mitchell et al., 2014; Maki et al., 2017). Because it has a narrow range of sorption preference due to limited access to interlayer surfaces in the clay, in this study, we have taken advantage of this limitation and enhanced the binding capabilities of these montmorillonites, especially for lipophilic chemicals such as BaP and aldicarb. To accomplish this, we amended parent montmorillonites with natural nutrients such as L-carnitine and choline, and investigated binding parameters and mechanisms for the polycyclic aromatic hydrocarbon, BaP and the pesticide, aldicarb. Computational chemistry models (Wang et al., 2017) suggest that L-carnitine and choline can bind strongly to the negative surfaces of interlayers and basal surfaces of montmorillonites due to the positive charges from quaternary ammonium groups on these nutrients.

Previous studies have shown that activated carbon is the most efficient adsorbent for BaP adsorption. However, the isothermal results for carnitine and choline amended montmorillonites showed a markedly improved binding that was saturable and fit the Langmuir equation. The binding capacity of these amended clays was 0.09 mol/kg, which was similar to the Q_{max} of activated carbon (0.1 mol/kg). These are the first clay-based materials discovered with high binding capacities and affinities for BaP that are comparable to activated carbon. Importantly, the base clay and amendments have shown to be safe for human and animal consumption, unlike carbon. This is consistent with our hydra data where activated carbon produced less protection against BaP than amended

montmorillonites and talc. Our earlier data suggested that the carnitine and choline replaced hydrated inorganic cations (mainly sodium and calcium) in parent montmorillonite and balanced platelet surface charge, exposing hydrophobic sites that favored organophilic chemical adsorption.

Talc is commonly used in baby powder, food and multiple industries, such as plastics, ceramics, paint, paper, and others. Based on our isothermal results, talc was the most efficient adsorbent for BaP due to its high binding capacity (Q_{max} = 0.2 mol/kg) and affinity.

The protective roles of carnitine and choline amended clays and talc were identified using the adult hydra assay with an inclusion of MAP to metabolize BaP. As indicated in the results, amended clays at an inclusion rate of 0.2% resulted in significant protection of hydra against BaP toxicity (with MAP). The *in vivo* protection is in alignment with *in vitro* isotherm results. Whereas, at the same inclusion levels for activated carbon, only minor protection was shown. Since activated carbon showed binding from isothermal analysis, the minimal protection of hydra by carbon is possibly due to the toxic effects of activated carbon at high inclusion concentrations, i.e., 0.2%. This result indicates that adsorbent amendment with carnitine and choline and talc are safe and result in very effective protection of adult hydra against BaP.

Besides binding capacity and affinity, enthalpy is also an important parameter indicating thermodynamic properties of the adsorption. We conducted isotherms at ambient and high temperatures and compared different K_d values derived at each temperature. Enthalpies calculated from the Van't Hoff equation were -42 kJ/mol for the

montmorillonite-carnitine and -46 kJ/mol for the montmorillonite-choline clays. The minus symbol suggests that the reaction is spontaneous in the forward direction to form toxin/adsorbent product. Since the absolute values were more than twice the minimum level for chemisorption (i.e., 20 kJ/mol), data suggest that the adsorption reaction is a chemisorption involving tight binding, not physisorption. Thus, carnitine and choline enhance the capacity, affinity and strength of binding for the BaP/clay complex.

To further investigate clay binding sites, amended adsorbents were heated at 800°C until the interlayers were dehydroxylated and collapsed. Figure 18 showed that binding capacities of the collapsed amended clays were slightly affected, suggesting that BaP has multiple binding sites on clay surfaces other than interlayers, including basal surfaces and edge sites. Many toxins, including aldicarb, have been shown to bind in the interlayer of clays (Tapp and Stotzky, 1995; Phillips, 1999; Brightsmith et al., 2008), but not BaP. Therefore, this binding of BaP to porous surfaces on these amended clays suggest that montmorillonite-carnitine/choline may sorb combinations of BaP and other toxins that require interlayers for binding with limited interference.

Aldicarb is a carbamate pesticide and previously only activated carbon has been shown to bind it effectively. Isotherms for aldicarb showed an increased binding on carnitine and choline amended clays compared to the parent montmorillonite. However, the affinity for adsorption (K_d values) were decreased upon dehydroxylation and collapse of the layers, suggesting that aldicarb binds mainly within the interlayer of clay surfaces, unlike BaP which binds to basal surfaces. The *in vivo* hydra bioassay confirmed that these amended clays were safe and effective, with significant and complete protection against 1

ppm aldicarb at the 0.1% inclusion level. Since carnitine and choline amended montmorillonites have been shown to bind terbuthylazine (a triazine herbicide) and diuron (a phenylurea pesticide), it is possible that these amended clays have the ability to bind similar chemicals such as the carbamates, triazines, and phenylurea classes of pesticides.

3.5 Conclusions

In summary, amending montmorillonite with carnitine and choline significantly increased the binding capacity, affinity and enthalpy for BaP and aldicarb. Since carnitine and choline amended clays were previously reported to bind aflatoxin and the herbicides (terbuthylazine and diuron), it further supports their potential use as "broad-acting" adsorbents to mitigate unintended exposures of humans and animals to complex mixtures of hazardous environmental contaminants. Other natural products, such as talc, showed higher capacity and affinity for BaP than other adsorbents, suggesting that it may also serve as a component in a mixture of broad-acting adsorbents to mitigate exposures from contaminated food and water. Importantly, enterosorbent therapy could be delivered in nutritional supplements, foods and snacks, animal feeds and stirred in flavored drinking water during emergencies and disasters.

4. DEVELOPMENT OF ENTEROSORBENTS THAT CAN BE ADDED TO FOOD AND WATER TO REDUCE TOXIN EXPOSURES DURING DISASTERS*

4.1 Introduction

People and animals can be unintentionally exposed to mixtures of hazardous chemicals through contaminated food and water supplies during droughts and floods. Food is susceptible to contaminants during droughts and extended periods of heat, when fungi can reach their optimal growth conditions for the production of mycotoxins (Grant and Phillips, 1998). Global warming promotes drought-stressed crops and mold growth, thus enhancing the threat of high-level mycotoxin contamination of the food supply. Moreover, the consumption of these toxins in the diet can increase incidence of disease and lethality (especially in the young) during outbreaks. Among the most important toxic mycotoxins occurring in food, aflatoxin B_1 (AfB_1) and zearalenone (ZEN) are widely distributed in cereal crops, including corn, barley, oats, peanuts and wheat. During extended periods of drought, humans and animals consuming mycotoxin-contaminated diets are vulnerable to the adverse effects of these toxins, including growth stunting, immunosuppression, hepatotoxicity, reproductive defects, cancer and death (Avantaggiato et al., 2014; Lemke et al., 1998; Murugesan et al., 2015).

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During disasters (such as hurricanes and floods), water and food can be contaminated with common pesticides such as glyphosate (among other contaminants). Glyphosate is one of the most frequently used herbicides to control weeds. It has been detected in air during spraying, as well as in water and food. Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption (Duke and Powles, 2008). Glyphosate is a contemporary issue due to its widespread use, distribution and potential carcinogenicity, including the risk of non-Hodgkin lymphoma and other haematopoietic cancers (Guyton et al., 2015a, b; IARC, 2015). Glyphosate's mode of action involves inhibition of enzymes associated with the synthesis of three amino acids: tyrosine, tryptophan and phenylalanine. Therefore, it is effective on actively growing plants, but not as a pre-emergence herbicide. The molecular space-filling models for each of these chemicals are shown in Figure 23. They were energy-minimized using a computational quantum mechanical AM1 method (Hyperchem 8.0) (Phillips et al., 1995).

Previously, our laboratory has conducted extensive intervention trials showing that montmorillonite clays, when included in the diet, are able to decrease aflatoxin exposure, toxicity and lethality in young animals and significantly reduce biomarkers of aflatoxin exposure from blood and urine in humans (Awuor et al., 2017; Maki et al., 2017; Mitchell et al., 2014). The mechanism for this protection involves tight adsorption of toxins into pores and onto active surfaces of sorbents within the gastrointestinal tract, resulting in decreased toxin bioavailability and toxicity (Phillips, 1999). Additionally, these studies have shown that the inclusion of montmorillonite clay had no impact on the palatability (texture, taste and odor) nor acceptability of the diet (Awuor et al., 2017). As indicated

above, montmorillonite clays are very effective binders for aflatoxin, but have very limited ability to adsorb other toxins. Currently, no sorbents have been shown to decrease the bioavailability of ZEN from contaminated diets, or glyphosate from contaminated water and food in humans and animals. Previously, a variety of sorbents have been tested for ZEN and glyphosate binding in vitro; however, our work is the first to investigate the efficacy of a potential enterosorbent for these toxins using a living organism. To address this problem, our research has focused on the development of novel, clay-based sorbents containing active surfaces that are broad-acting for a variety of important toxins, such as AfB_1, ZEN and glyphosate. Based on previous literature, the treatment of clays, such as montmorillonites, with acid results in the exchange of interlayer cations with protons from the acid, following the partial dissociation of octahedral and tetrahedral sheets in the clay structure. The final reaction product of acid processed montmorillonite clays (APMs) is thought to be a mixture of delaminated parent clay layers containing chains of amorphous silica on the edges, amorphous silica, and cross-linked silica (Figure 24) (Komadel et al., 1990; Madejova et al., 1998; Tyago et al., 2006). The pH of the final APM product is approximately 3, which is similar to acidic foods such as meat, cheese, and chocolate. In fact, the inclusion of acidic food additives can serve as a common taste enhancer and increase palatability and consumption (Deshpande et al., 2015). Also, acids, such as sulfuric acid, are permitted as food and feed additives to adjust pH (FDA, 2018a, b). Thus, the inclusion of APMs at a low concentration in the diet of humans and animals for short-term treatments should be safe. A variety of acid processed clays have already been developed and used extensively for bleaching purposes (De et al., 2009), removal of plant

pigments from oils (Yip et al., 2005), and sequestering various organic and inorganic contaminants from water during decontamination and purification procedures (Ake et al., 2001; Resmi et al., 2012; Ugochukwu and Fialips, 2017). However, there are no reports suggesting that these types of materials (APMs) might be included in the diets of animals and humans for short-term treatment to decrease exposure to toxins from contaminated water and food.

To determine the binding efficacy of APMs, we conducted equilibrium isothermal analyses and dosimetry studies to derive binding parameters and gain insight into: 1) surface capacity and affinity, 2) potential mechanisms of sorption, 3) thermodynamics of toxin/surface interactions, and 4) estimated dose of sorbent required to maintain threshold limits. We have also used a toxin-sensitive living organism (Hydra vulgaris) to predict the in vivo safety and efficacy of APMs and their ability to mitigate the toxicity of commonly occurring mycotoxins and pesticides.

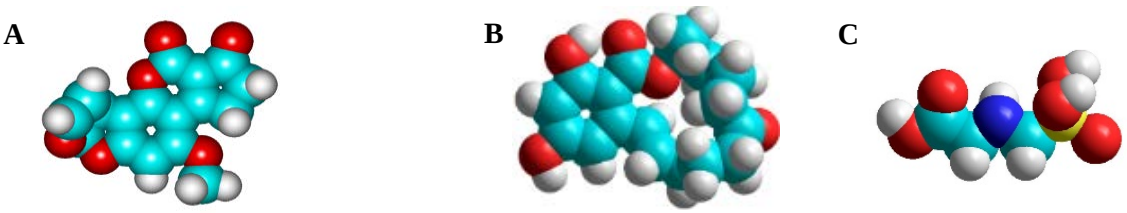

Figure 23. Chemical structures and molecular models of AfB_1 (A), ZEN (B), and glyphosate (C), illustrating spatial orientation and size of functional groups.

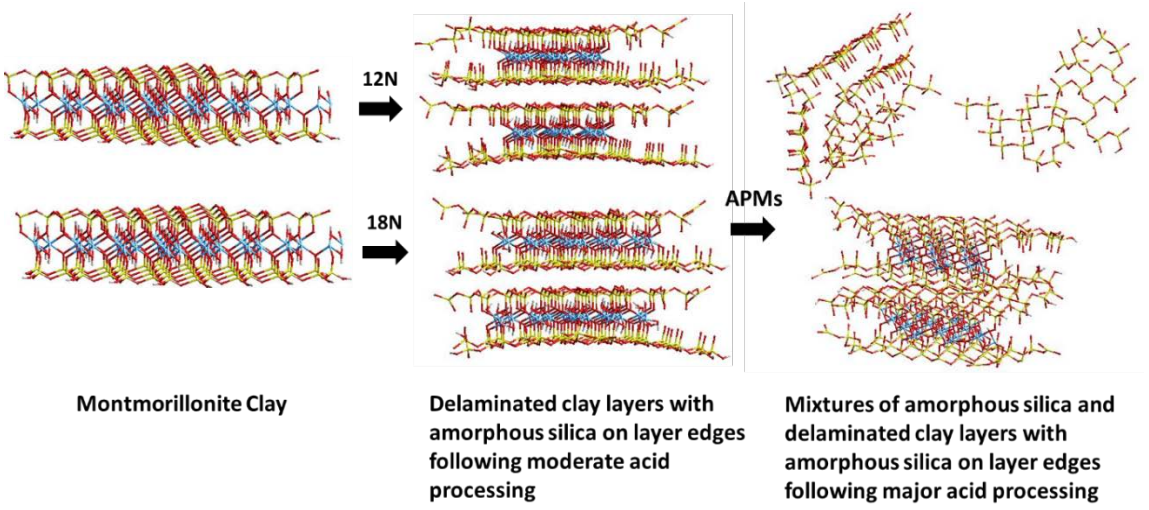

Figure 24. Energy minimized molecular models of parent montmorillonite clay versus moderately and highly processed APMs, illustrating the process and potential products of acid treatment.

4.2 Materials and methods

4.2.1 Reagents

High Pressure Liquid Chromatography (HPLC) grade acetonitrile, reagents and pH buffers (4.0, 7.0 and 10.0) were purchased from VWR (Atlanta, GA). AfB_1, ZEN and glyphosate were purchased from Sigma Aldrich (Saint Louis, MO). Sulfuric acid (H_2SO_4, 95-98%) and formic acid (HCOOH, 88%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). In this study, a calcium montmorillonite (CM) was obtained from Engelhard Corp (Cleveland, OH) with an average total surface area as high as 850 m^2/g, an external surface area of approximately 70 m^2/g and cation exchange capacity equal to 97 cmol/kg (Grant and Phillips, 1998). Sodium montmorillonite (SM) was a gift from the Source Clay Minerals Repository at the University of Missouri-Columbia with cation

exchange capacity equal to 75 cmol/kg. The generic formula for these clays is: $(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$. Samples of both clays contain some quartz, mica, calcite, orthoclase feldspars and sanidine as impurities, and mesopores of approximately 5 nm in diameter (Marroquin-Cardona et al., 2011). Ultrapure deionized water (18.2 MΩ) was generated in the lab using an Elga™ automated filtration system (Woodridge, IL) and was used in all experiments.

4.2.2 Synthesis of sorbents

CM and SM were treated with sulfuric acid to produce broad-acting sorbents with high surface areas and porosities. In glass beakers, 5 g (6%) of CM and SM clay mineral suspensions were individually treated with 12N or 18N sulfuric acid. The solutions were vigorously stirred and kept in an oven at 60ºC overnight. The slurry was cooled, centrifuged at 2000 g for 20 min and washed thoroughly with distilled water. This centrifugation-washing process was repeated multiple times until the pH for each treatment group was constant. All samples were dried in the oven at 110ºC overnight before grinding and sieving through a 125 µm screen. These grinding and sieving steps were necessary to obtain clay particles of uniform size (Neji et al., 2011; Wang et al., 2017).

To investigate the binding of toxins to sorbent surfaces and to determine the importance of intact clay interlayers, experiments with heat-collapsed sorbents were also conducted. Collapsed sorbents were prepared by heating APMs at 200ºC for 30 min followed by 800ºC for 1 h to collapse the interlayer (Loffredo et al., 2014).

4.2.3 In vitro isothermal adsorption

The toxin stock solution was prepared by dissolving pure crystals into acetonitrile. Aliquots of the solution were injected into a sample of distilled water (pH 6.5) for each toxin to yield 8 ppm (8 µg/mL) AfB_1, 4 ppm ZEN and 10 ppm glyphosate solutions. The concentrations were established based on the octanol-water partitioning coefficients (K_{ow}) so that precipitation during the testing was not a factor, and the optimal toxin/sorbent ratio was designed to fit the Langmuir model. Then 0.002% (w/v) of sorbents were exposed to an increasing concentration gradient of toxin in solution. In all assays, besides test samples, there were 3 controls consisting of distilled water, toxin solution without sorbent and sorbent solution without toxin. The control and test groups in disposable glass tubes were capped and agitated at 1000 rpm for 2 h at either 24ºC (T_1) or 37ºC (T_2) using an electric shaker. All samples were then centrifuged at 2000 g for 20 min to separate the sorbent/toxin complex from solution. The UV-visible scanning spectrophotometer was used to scan and read the adsorption peak at 362 nm for AfB_1 and 236 nm for ZEN (Grant and Phillips, 1998; Lemke et al., 1998).

Glyphosate was analyzed using a Waters Acquity® LC/MS/MS equipped with a BEH C18 column (50 x 2.1 mm). Separation was obtained with a mobile phase of water with 0.1% formic acid (eluate A) and acetonitrile with 0.1% formic acid (eluate B) (5%-100% of eluate B in 10 min) at 0.3 mL/min with a 40 µL injection volume and a negative electrospray ionization mode at 4.5 kV spray voltage. Nitrogen gas was used as the collision gas and curtain gas, and argon gas was used as the nebulizer gas and heater gas. The source temperature was kept at 225ºC. The mass spectrometer was operated under

multiple reaction monitoring (MRM) mode and the monitored precursor and product ions were 168 and 63/81. The unit mass resolution was used for the ion mass analyzer. Empower analyst software was used to control the LC/MS/MS system and acquire the data (Mazumder and Sasmal, 2001). To validate this detection method for glyphosate, a standard solution of glyphosate was prepared in distilled water at concentrations between 20 ppm and 0.5 ppm to measure the standard curves. The standard curve for glyphosate is linear ($r^2 > 0.99$), and described by the equation $y = 32185x + 41034$, where: x is the glyphosate concentration (ppm) and y is the signal intensity from LC/MS/MS.

4.2.4 Data calculations and curve fitting

Glyphosate concentrations in solution were detected by LC/MS/MS and calculated from peak area. A UV-visible scanning spectrophotometer was used to calculate the concentration of AfB_1 and ZEN left in solution (c) using Beer's law.

Beer's law Absorbance = ε L c

Where: ε is the molar extinction coefficient (ε for AfB_1 = 21,865 $cm^{-1}mol^{-1}$, ε for ZEN = 24,833 $cm^{-1}mol^{-1}$), L is the path length of the cell holder = 1 cm.

The amount adsorbed for each data point was calculated from the concentration difference between test and control groups. More specifically, the y-axis is the amount of toxin bound by sorbents (in mol/kg). It is calculated by the difference in moles of free toxin in the test solution versus control groups and is then divided by the mass of the sorbents included. These data were then plotted using Table-Curve 2D and a computer program that was developed with Microsoft Excel to derive values for the variable parameters. The best fit for the data was a Langmuir model, which was used to plot

equilibrium isotherms from triplicate analysis. The isotherm equation was entered as user-defined functions:

Langmuir model $q = \text{Qmax}\ (\frac{KdCw}{1+KdCw})$

Where: q = toxin adsorbed (mol/kg), Q_{max} = maximum capacity (mol/kg), K_d = distribution constant, C_w = equilibrium concentration of toxin.

The plot will normally display a break in the curve. The value on the x-axis where the curve breaks is an estimate of K_d^{-1}. The value on the y-axis where the curve breaks is an estimate of Q_{max}. The definition of K_d is derived from the Langmuir equation giving:

$$Kd = \frac{\text{q}}{(\text{Qmax} - \text{q})\text{Cw}}$$

The enthalpy (ΔH) was calculated by comparing the difference of K_d values at 24 C (T_1) and 37 C (T_2) by the following equation:

Van't Hoff equation $$\Delta Hads = \frac{-\text{Rln}(\frac{\text{Kd2}}{\text{Kd1}})}{(\frac{1}{\text{T2}})-(\frac{1}{\text{T1}})}$$

Where: R (ideal gas constant) = 8.314 J mol^{-1} K^{-1}, T = absolute temperature (K).

4.2.5 Dose of sorbents required to maintain threshold limits of toxins

In the dosimetry study, ZEN and glyphosate were diluted with distilled water from a stock solution to derive 2 ppm ZEN and 20 ppm glyphosate solutions that were set at twice their threshold limits (ZEN in food = 1 ppm; glyphosate in water and food = 10 ppm) (IRIS, 1987). An increasing gradient of sorbent inclusion was added to 5.0 mL of ZEN solution at 0.005, 0.02, 0.05, 0.2, 1.0 mg/mL, and glyphosate at 0.02, 0.1, 0.5, 1.0, 2.0 mg/mL. Control groups included distilled water and toxin solution without sorbent. Both control and test groups in disposable glass tubes were capped and agitated at 1000

rpm for 2 h. All samples were then centrifuged at 2000 g for 20 min. Aliquots of ZEN were read on a UV-visible scanning spectrophotometer at 236 nm and calculated by Beer's law for the remaining (unbound) ZEN concentration. Aliquots of glyphosate were measured by LC/MS/MS and calculated by the signal peak area for the remaining glyphosate concentration. The toxin sorption percentage was calculated by the difference between control and test groups. Predicted algorithms were derived to extrapolate the sorbent inclusion levels required to meet the threshold limits for ZEN in food and glyphosate in water and food.

4.2.6 Hydra bioassay

Hydra vulgaris were obtained from Environment Canada (Montreal) and maintained at 18ºC. The hydra classification method was used with modification (Ottinger et al., 1999) to rate morphology of the adult hydra as an indicator of toxicity. Mature and non-budding hydra in similar sizes were chosen for testing in order to minimize differences between samples. Toxin treatment groups included 20 ppm AfB_1, 4 ppm ZEN and 30 ppm glyphosate in the hydra media based on the minimum effective dose of each toxin resulting in 100% mortality in 92 h. Toxin mixture treatment groups included 1 ppm AfB_1 and 6 ppm ZEN based on the ratio of average concentrations of AfB_1 and ZEN reported worldwide in animal feedstuffs (Rhodes and Brown, 1993). The combination of the two mycotoxins (ZEN and AfB_1) was tested in the hydra bioassay, since these mycotoxins tend to commonly occur in food and feed together, especially during outbreaks of drought. All test solution tubes were capped and prepared by shaking at 1000 rpm for 2 h and centrifugation at 2000 g for 20 min prior to exposure of hydra in pyrex

dishes to toxin. For each sample, three hydra were included into 4 mL of test media and kept at 18ºC. Hydra solutions were not changed throughout the 92 h testing period. The assay included monitoring times at shorter intervals during the first two days (0, 4, 20, and 28 h) and 24 h intervals for the last three days (44, 68, and 92 h) (Ozcan and Ozcan, 2004). The hydra morphological response was scored and recorded after exposure to toxin, with and without sorbent treatment. The toxicity rating was determined by calculating the average score for morphological changes for a certain group at a specific time point.

4.2.7 Statistical analysis

A two-way t-test was used to calculate statistical significance. Each experiment was triplicated to derive an average and standard deviation. In the t-test, Q_{max} from heat collapse analyses and toxicity scores from the hydra bioassay were included to calculate t-values. The t-value and degrees of freedom were compared in a p-value table to determine the statistical significance. Results were considered significant at $p \leq 0.05$.

4.3 Results and discussion

Figure 23 illustrates the chemical structures and molecular models of AfB_1 (A), ZEN (B), and glyphosate (C). Figure 24 shows the energy minimized molecular models of parent montmorillonite clay versus moderately and highly processed APMs, illustrating the process and the products of acid treatment.

Equilibrium isotherms were generated by Table-Curve 2D and a computer program developed in our laboratory using Microsoft Excel to derive affinities (K_d), capacities (Q_{max}) and the enthalpies (ΔH) of sorption for toxin-surface interactions. Figure 25A shows the isothermal plot for AfB_1 on the surfaces of acid processed CM (APCM).

Figure 25B shows the isothermal plot for AfB_1 on the surfaces of acid processed SM (APSM). The r^2 values (> 0.8) for the Langmuir model and saturable plots indicate that AfB_1 binds tightly onto clay surfaces and does not dissociate easily. The derived Q_{max} values indicated that APMs were able to bind AfB_1 like the parent clays. Based on previous studies, sorbents such as SM had high expansibility in water with enhanced access to active surfaces resulting in a higher K_d for AfB_1 versus APSM. To calculate the sorption enthalpies of APM clays for AfB_1, thermodynamic isotherms were run at 2 different temperatures, i.e., 24ºC (T_1) and 37ºC (T_2). Calculated enthalpies (ΔH) for APCM-12N, APCM-18N, APSM-12N and APSM-18N were equal to -100 kJ/mol, -67 kJ/mol, -135 kJ/mol and -131 kJ/mol, respectively (Figure 25C and D). These findings indicate that AfB_1 was chemisorbed tightly to the clay surfaces and it is consistent with the Langmuir model for the interaction. The minus symbol suggests that the reaction is spontaneous in the forward direction to form toxin/adsorbent product. Since the absolute values were more than the minimum level for chemisorption (i.e., 20 kJ/mol), data suggest that the adsorption reaction is a chemisorption involving tight binding, not physisorption. The safety and efficacy of sorbents were further confirmed using the hydra bioassay (Figure 25E). Following the inclusion of CM and APCM at a 0.005% inclusion level, adult hydra were completely protected from aflatoxin toxicity with no morphological and physiological changes from the hydra media control group.

Figure 26 shows the isotherms for ZEN adsorption on the surfaces of APCM and APSM, respectively. Freundlich isotherms in Figure 26A and B suggest that ZEN adsorption onto the surfaces of parent CM and SM clays shows a linear trend, which

indicates partitioning activity, instead of tight binding at saturable sites. APMs were able to improve ZEN binding with saturable curves that fit the Langmuir model ($r^2 > 0.8$), which indicate tight binding onto active clay surfaces. The high binding capacity ($Q_{max} > 0.2$) suggests the ability of APMs to serve as effective ZEN enterosorbents; this effect is probably due to higher surface areas (and more porosity) than that of parent clays. The hydra data in Figure 4C shows the significantly enhanced binding of ZEN onto APM surfaces at an inclusion rate of 0.01% that resulted in complete protection against ZEN toxicity. The parent clay (CM) showed no protection against ZEN toxicity at the same inclusion rate.

The APMs were collapsed with heat to determine the importance of the interlayer space for AfB_1 and ZEN sorption, singly and in combination. After heating clays at 800ºC, the interlayers were dehydroxylated and collapsed. Figure 27A shows that binding capacities for AfB_1 on collapsed APMs were significantly reduced, with 20% and 14% of aflatoxin remaining on collapsed (Co) APCM-12N and APCM-18N, respectively. This dramatic decrease of AfB_1 suggests that more than half of the AfB_1 binds within the intact interlayers available in the processed sorbents as predicted from thermodynamic calculations and computational modeling. Based on the partial positive charge on carbons C11 and C1 of the AfB_1 dicarbonyl system and the strength of adsorption of planar analogs and derivatives of AfB_1, an electron donor acceptor mechanism was postulated for the AfB_1 sorption mechanism onto the negatively charged montmorillonite interlayer. On the other hand, Figure 27B shows that the percentages of the ZEN remaining on collapsed clays were 50% and 63% (i.e. CoAPCM-12N and CoAPCM-18N, respectively). Unlike

AfB_1, less than half of the ZEN binds within the intact interlayers in the processed sorbents indicating the potential for significant binding to sites other than the interlayer. This difference in binding sites for ZEN is possibly due to its hydrophobic nature with a log P (octanol-water) equal to 3.6 and neutral charge. Despite this difference in binding sites, both the adsorption isotherms of AfB_1 and ZEN indicate tight binding and the binding site is saturable with toxins. To investigate the ability of APMs to protect against combinations of AfB_1 and ZEN, adult hydra were exposed to a common toxin mixture of 1 ppm AfB_1 and 6 ppm ZEN, based on an worldwide average AfB_1 and ZEN concentrations reported in animal feedstuffs (Figure 27C). The inclusion of APMs at 0.1% w/w significantly prevented the mortality of hydra with 90% and 100% protection from APCM-12N and APCM-18N, respectively. The slight protection by parent CM is in alignment with the *in vitro* isothermal results indicating that the parent CM can bind AfB_1, but not ZEN. APMs were shown to significantly protect the hydra from AfB_1 and ZEN confirming simultaneous sorption of both toxins, with limited interference. Importantly, this finding suggests that APMs may be able to decrease exposures to foodborne toxins in humans and animals when included in the diet.

The widespread occurrence of ZEN in food and feed is commonly associated with severe drought and adverse impacts to health. There are no clay-based sorbents, or other treatments, to prevent disease and death from this toxin. Thus, to develop an optimal toxin enterosorbent for ZEN, it is important to gain insight into the binding efficacy and the thermodynamics of the interaction of this toxin on active surfaces of APMs. To calculate the binding enthalpy of APMs for ZEN, isotherms for ZEN were run at 24ºC and 37ºC.

Calculated enthalpies (ΔH) for APCM-12N, APCM-18N, APSM-12N and APSM-18N were equal to -90 kJ/mol, -75 kJ/mol, -74 kJ/mol, and -78 kJ/mol, respectively (Figure 28A and 28B). Based on the minus symbol and the high absolute values (higher than 20 kJ/mol), data suggest that the adsorption reaction is a chemisorption involving tight binding, not physisorption

Additionally, we conducted a study to determine the dose of APMs that could be used to treat humans and animals during droughts and outbreaks of ZEN toxicosis. The regulatory threshold for ZEN in food in the UNITED STATES is equal to 1.0 ppm. Based on our dosimetry results (Figure 28C), the predicted inclusion rates were equal to 1.0 g/kg and 0.73 g/kg (w/w of diet) for APCM-12N and APCM-18N, respectively. These results suggest that a low dose of APMs would be required for treatment and could serve as an effective sorbent to decrease ZEN exposure when administered before each meal. This low inclusion rate was derived from the isothermal results and confirmed in the hydra bioassay. This information will help to facilitate a determination of dosage requirements for enterosorbent treatment in animals and humans for ZEN during outbreaks.

Glyphosate is one of the most widely used herbicides and has been detected in water and food. During hurricanes and floods, glyphosate (and other environmental chemicals) may be mobilized and redistributed from soil and sediment at the site of these disasters into vulnerable communities. Therefore, strategies to reduce unintentional exposures of humans and animals to glyphosate (and other) potential carcinogens [7] during disasters are warranted.

Our isothermal results in Figure 29A and B showed significantly increased glyphosate binding capacities for APMs compared to the parent clays. This high binding efficacy is further confirmed by the hydra bioassay (Figure 29C and D), where APMs at the inclusion rate at 0.1% were able to completely protect hydra against glyphosate toxicity, whereas the parent SM was shown to have limited protection. The results of a thermodynamic study of glyphosate adsorption in Figure 30A showed binding enthalpies of SM, APSM-12N and APSM-18N equal to -36 kJ/mol, -37 kJ/mol and -22 kJ/mol, respectively. This finding indicates that the adsorption of glyphosate is spontaneous in the forward direction and involves chemisorption, or tight binding onto active clay surfaces. To determine the dose to maintain a threshold limit for glyphosate and facilitate the decision of dosage forms for treatment, a dosimetry study was conducted (Figure 30B). In this study, a NOEL (no observed effect level) in food and water was used as the threshold limit for glyphosate due to its probable carcinogenicity. Based on our results, the required doses for APCM-12N and APCM-18N were 2 g/kg and 1.38 g/kg, suggesting increased binding efficacy compared to the predicted dose for CM (i.e. 3.6 g/kg). Assuming 1 kg of diet is consumed by adults at each meal, then small capsules of APMs, or snacks, vitamins and flavored water containing APMs could be administered before each meal to significantly reduce glyphosate exposure during a disaster. Additionally, APMs may also be added to community garden soils (before and after disasters) to reduce the potential for toxin translocation to plants and dermal exposures from contaminated soil.

APMs had high binding capacity and affinity for AfB_1, ZEN and glyphosate and resulted in complete protection against individual toxins as well as mycotoxin mixtures.

With toxin exposure, no morphological and physiological changes were observed in the APM treatment groups. The dose of APMs required to protect hydra and meet the threshold limit for ZEN and glyphosate was lower than the parent clays they were derived from. These findings were predicted by the increased binding capacity for ZEN and glyphosate *in vitro* based on equilibrium isotherms. The mechanisms of APM sorption of toxins likely involve interactions at multiple binding sites on active surfaces of APMs, including partially delaminated clay layers, amorphous silica and cross-linked silica. The heterogeneity of APM surfaces were consistent with a lower correlation ($r^2 > 0.8$) for a homogenous Langmuir plot of the data. The fact that the Langmuir model remained the best fit for the adsorption curves of AfB_1, ZEN and glyphosate, indicated that there was a major binding site with high affinity for each chemical. Earlier work suggested that acid processing of montmorillonite clay significantly increased pore volume and pore diameter resulting in higher surface areas than the parent clay (Ozcan and Ozcan, 2004). This study is consistent with these findings in that the total surface area of APMs was increased by 60% from approximately 800 m^2/g (for parent clays) to 1300 m^2/g (for APM-18N). This increased surface area and porosity was consistent with the broad-acting ability of APMs to bind different toxins. Previous studies have reported that the surfaces of acid processed bentonites (similar to APMs) have both positive and negative charges. These charged surfaces may be partially responsible for the high binding efficacy for neutral chemicals like AfB_1 and ZEN, or zwitterionic chemicals like glyphosate.

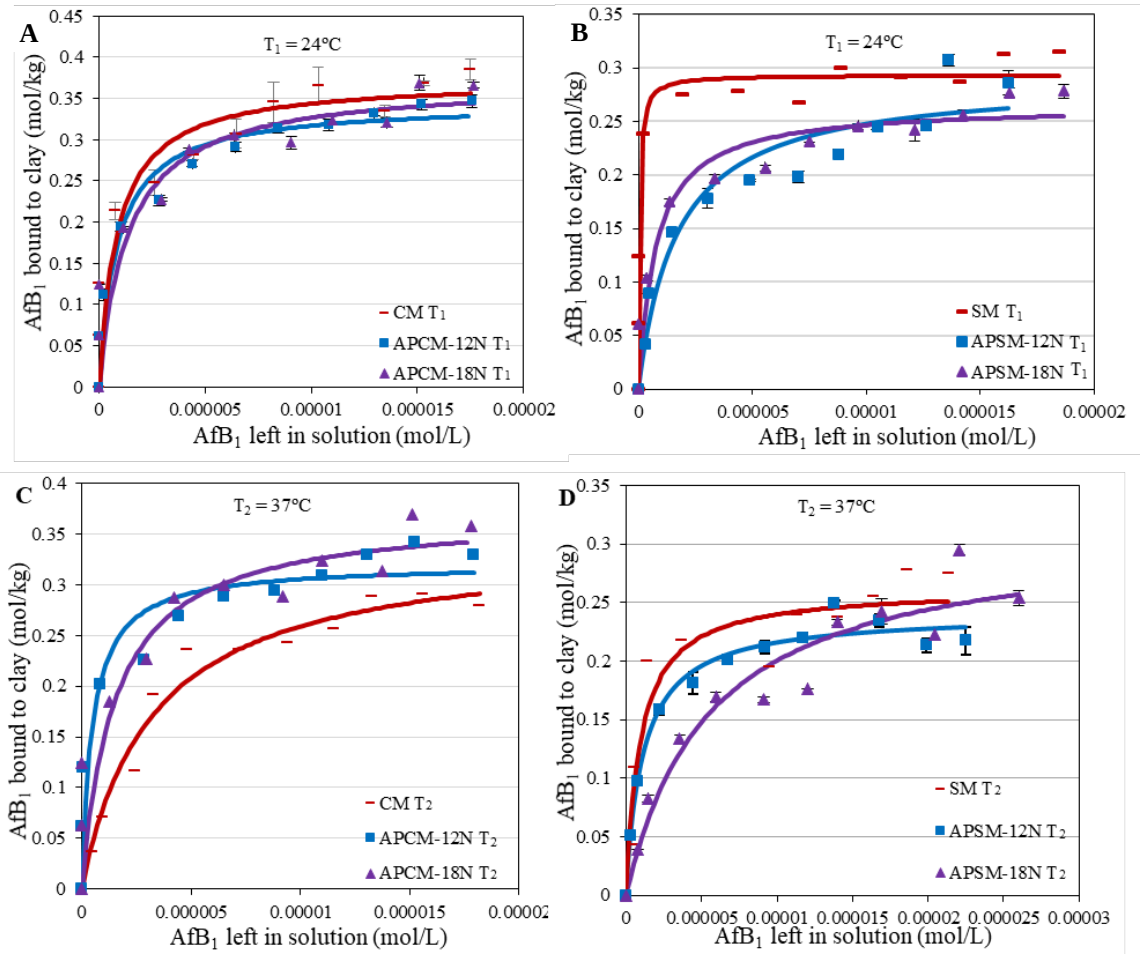

Figure 25. Langmuir plots of AfB_1 on APCM and APSM versus parent clays showing the observed and predicted Q_{max} values at 24ºC (T_1) in A and B and 37ºC (T_2) in C and D. The Q_{max} values indicated tight binding. Hydra toxicity and protection by CM and APCM at 0.005% inclusion level against 20 ppm AfB_1 (E). Hydra media and toxin controls are included for comparison. The score of 10 (indicating no toxicity) contains the hydra media and both APCM treatments (* $p \leq 0.05$, ** $p \leq 0.01$). The binding and affinity parameters are as follows: (A) CM T_1: Q_{max} = 0.37; K_d = 1E6; APCM-12N T_1: Q_{max} = 0.34; K_d = 1E6; APCM-18N T_1: Q_{max} = 0.37; K_d = 8E5. (B) SM T_1: Q_{max} = 0.3; K_d = 2E7; APSM-12N T_1: Q_{max} = 0.29; K_d = 6E6; APSM -18N T_1: Q_{max} = 0.27; K_d = 2E6. (C) CM T_2: Q_{max} = 0.34; K_d = 3E5; APCM-12N T_2: Q_{max} = 0.35; K_d = 5E5; APCM-18N T_2: Q_{max} = 0.31; K_d = 3E5. (D) SM T_2: Q_{max} = 0.26; K_d = 1E6; APSM-12N T_2: Q_{max} = 0.24; K_d = 9E5; APSM-18N T_2: Q_{max} = 0.31; K_d = 2E5.

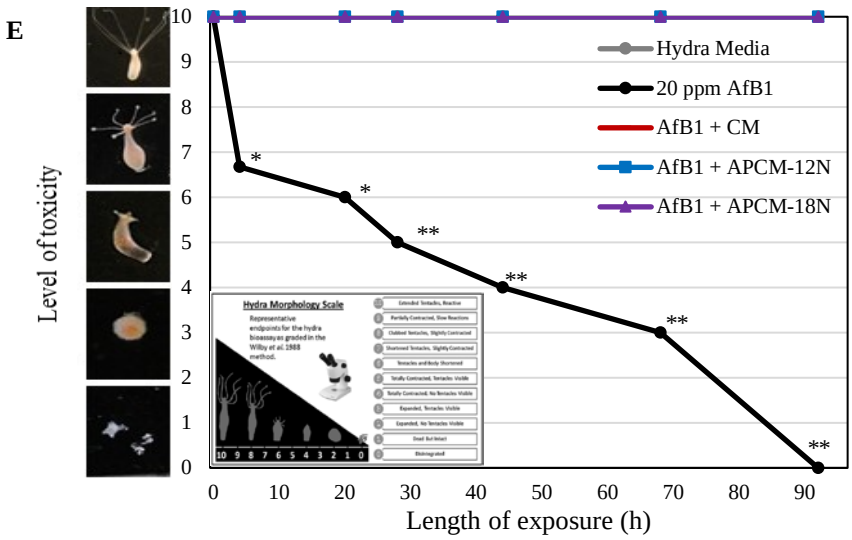

Figure 25 Continued.

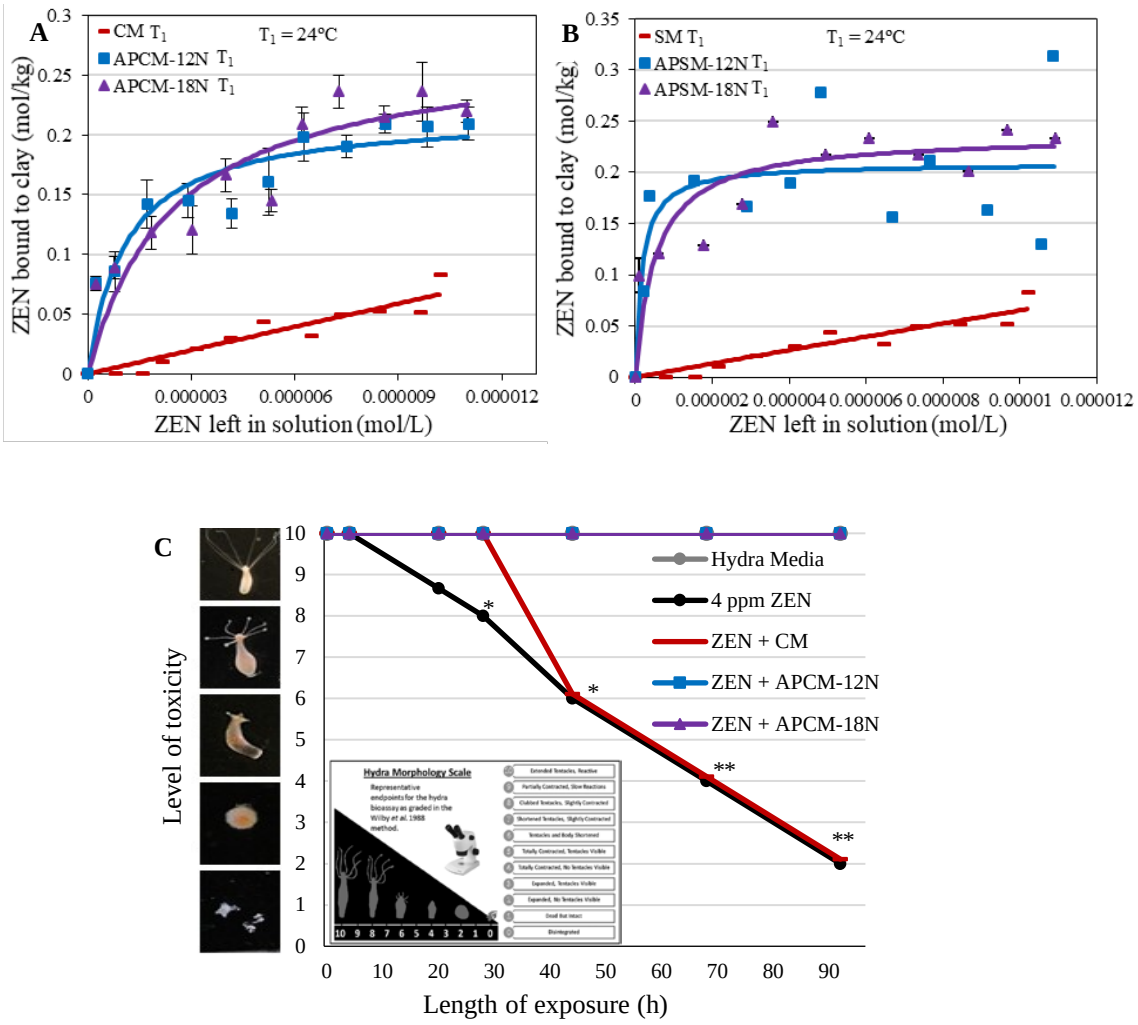

Figure 26. Langmuir plots of ZEN on APCM (A) and APSM (B) versus parent clays showing the predicted Q_{max} values at 24ºC (T_1). Hydra toxicity and protection by CM and APCM at 0.01% inclusion level against 4 ppm ZEN (C). Hydra media and toxin controls are included for comparison. The score of 10 (indicating no toxicity) contains the hydra media and both APCM treatments (* $p \leq 0.05$, ** $p \leq 0.01$). The binding and affinity parameters are as follows: (A) APCM-12N T_1: Q_{max} = 0.22; K_d = 1E6; APCM-18N T_1: Q_{max} = 0.28; K_d = 4E5. (B) APSM-12N T_1: Q_{max} = 0.21; K_d = 6E6; APSM-18N T_1: Q_{max} = 0.24; K_d = 2E6.

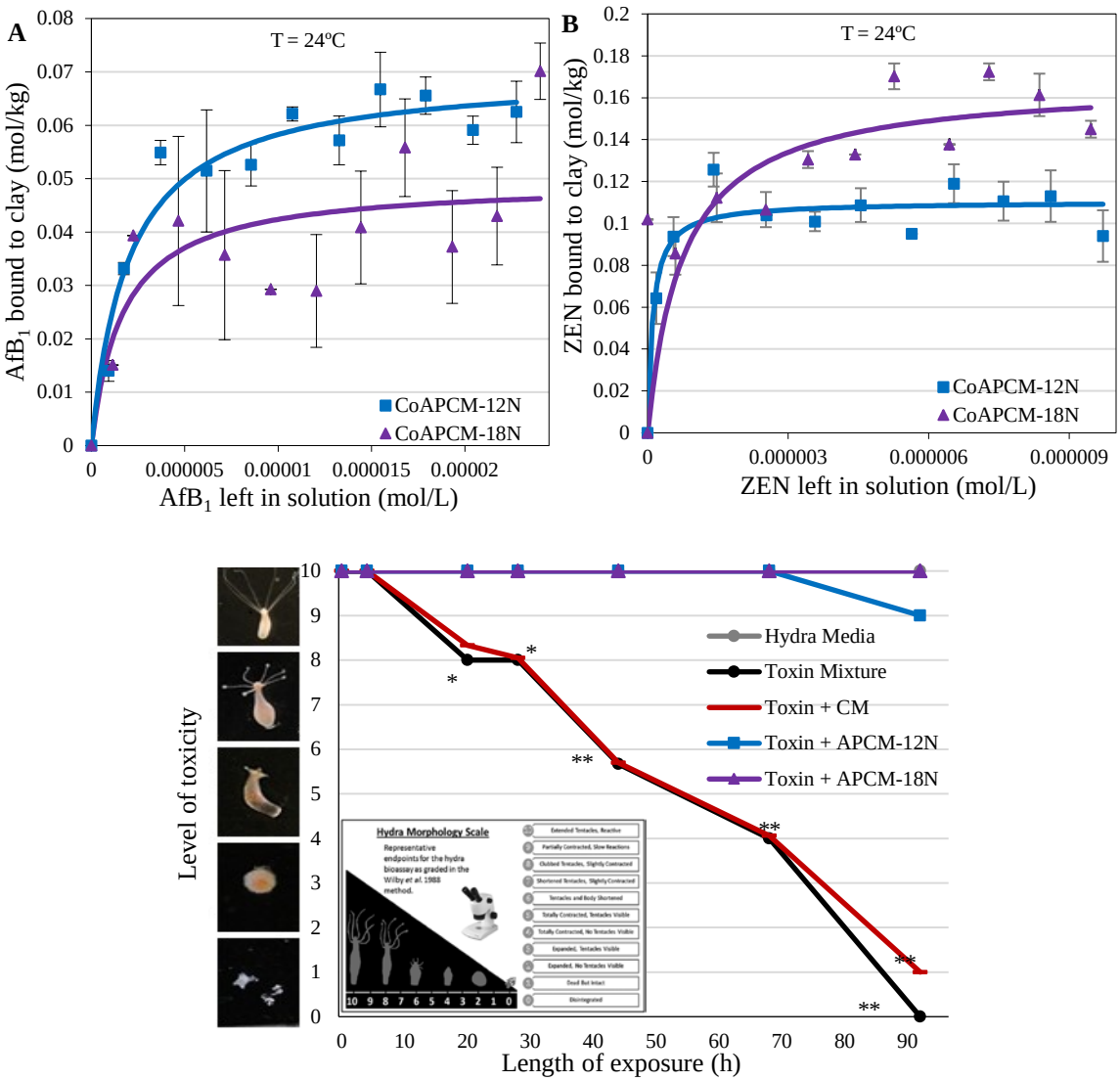

Figure 27. Langmuir plots of AfB_1 (A) and ZEN (B) on collapsed (co) APCM. The Q_{max} values indicated tight binding. Hydra toxicity and protection by CM and APCM at 0.1% inclusion level against the toxin mixture (6 ppm ZEN and 1 ppm AfB_1) (C). Hydra media and toxin controls are included for comparison. The score of 10 (indicating no toxicity) contains the hydra media and APCM-18N treatment (* $p \leq 0.05$, ** $p \leq 0.01$). The binding and affinity parameters are as follows: (A) CoAPCM-12N: Q_{max} = 0.07**; K_d = 5E5; CoAPCM-18N: Q_{max} = 0.05**; K_d = 6E5. (B) CoAPCM-12N: Q_{max} = 0.11*; K_d = 9E6; CoAPCM-18N: Q_{max} = 0.17*; K_d = 1E7.

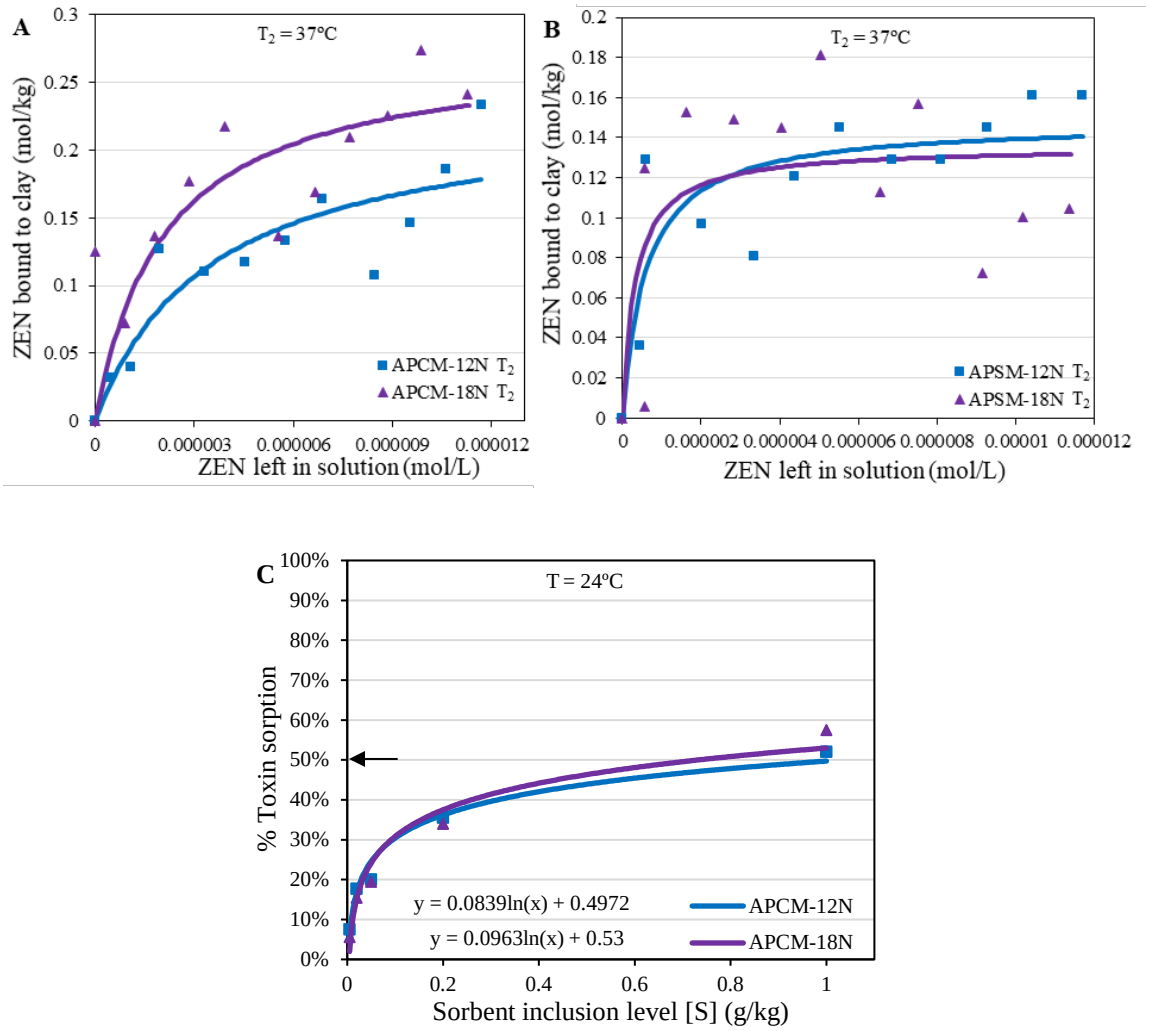

Figure 28. Langmuir plots of ZEN on APCM (A) and APSM (B) showing the predicted Q_{max} values at 37 C (T_2). Extrapolation of sorbent dosimetry for ZEN exposure (C). The binding and affinity parameters are as follows: (A) APCM-12N T_2: Q_{max} = 0.23; K_d = 4E5; APCM-18N T_2: Q_{max} = 0.28; K_d = 5E5. (B) APSM-12N T_2: Q_{max} = 0.15; K_d = 2E6; APSM-18N T_2: Q_{max} = 0.14; K_d = 3E6.

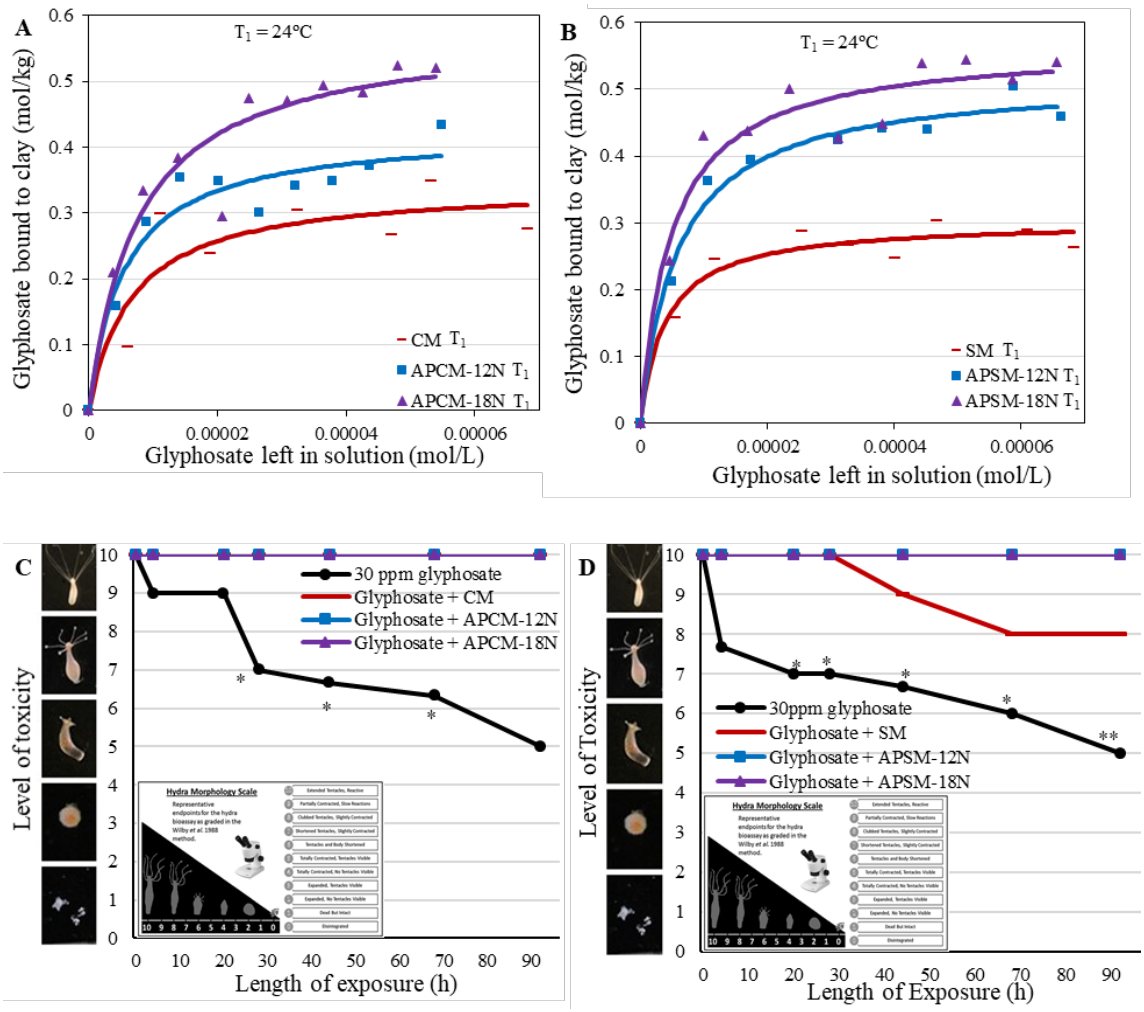

Figure 29. Langmuir plots of glyphosate on APCM (A) and APSM (B) versus parent clays showing the predicted Q_{max} values at 24ºC (T_1). Hydra toxicity and protection by CM and APCM (C), and SM and APSM (D) at a 0.1% inclusion rate are shown against 30 ppm glyphosate. Hydra media and toxin controls are included for comparison. The score of 10 (indicating no toxicity) contains the hydra media and all clay treatment groups except SM (* $p \leq 0.05$, ** $p \leq 0.01$). The binding and affinity parameters are as follows: (A) CM T_1: Q_{max} = 0.34; K_d = 2E5; APCM-12N T_1: Q_{max} = 0.42; K_d = 2E5; NSP-18N T_1: Q_{max} = 0.58; K_d = 1E5. (B) SM T_1: Q_{max} = 0.3; K_d = 3E5; APCM-12N T_1: Q_{max} = 0.52; K_d = 2E5; NSP-18N T_1: Q_{max} = 0.56; K_d = 2E5.

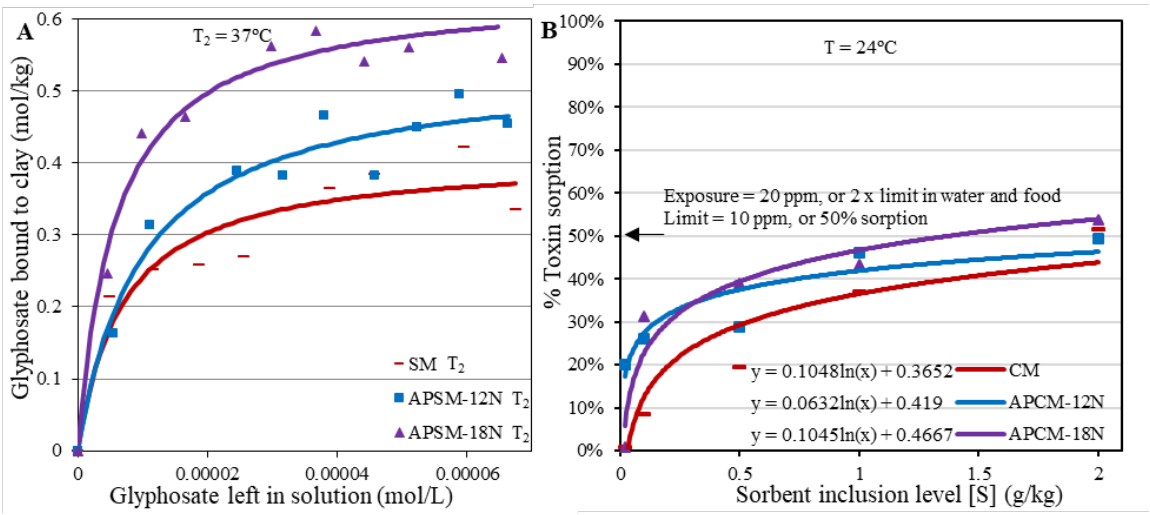

Figure 30. Langmuir plots of glyphosate on APSM showing the predicted Q_{max} values at 37ºC (T_2) (A). Extrapolation of sorbent dosimetry for glyphosate exposure (B). The binding and affinity parameters are as follows: (A) SM T_2: Q_{max} = 0.26; K_d = 2E5; APSM-12N T_2: Q_{max} = 0.53; K_d = 1E5; APSM-18N T_2: Q_{max} = 0.6; K_d = 2E5.

4.4 Conclusion

The main novelty of this study is the fact that we can utilize APMs that contain surface areas and porosities higher than parent clays as *broad-acting* toxin enterosorbents for multiple contaminants in food and water, especially at the site of disasters. Based on our previous studies, montmorillonite clay was the most effective aflatoxin sorbent and the only sorbent shown to be safe for human consumption when included in diets, but there are no reports of effective clay-based sorbents to reduce ZEN and glyphosate bioavailability *in vivo*. In this study, novel APMs were shown to bind AfB_1, ZEN and glyphosate with high binding capacity and enthalpy, and completely protected hydra against individual toxins and mycotoxin mixtures. We also estimated the dose of sorbent required to keep exposures below the regulatory threshold level for individual toxins,

which will help to determine an optimal dose for short-term treatment. Based on the toxin binding efficacy of APMs for AfB_1, ZEN and glyphosate (*in vitro* and *in vivo*), it is possible that these, and other acid processed clays, may be broad-acting in their ability to decrease exposures to numerous chemical contaminants from food and water such as other pesticides, PAHs, commercial solvents, plasticizers, and metals. We anticipate the short-term inclusion of broad-acting APMs in the diet of humans and animals as a protective measure to minimize unintended exposures from contaminated food and water supplies at the site of disasters, including droughts, hurricanes, floods, chemical spills, fires, and acts of terror.

5. STRONG ADSORPTION OF POLYCHLORINATED BIPHENYLS BY PROCESSED MONTMORILLONITE CLAYS: POTENTIAL APPLICATIONS AS TOXIN ENTEROSORBENTS DURING DISASTERS AND FLOODS*

5.1 Introduction

Polychlorinated biphenyls (PCBs) are complex mixtures of isomers and congeners that were marketed as commercial products based on their percentage of chlorine composition. Their physical and chemical properties such as low vapor pressure, low aqueous solubility, and inertness to water, acid, alkali and heat contribute to their extreme persistence in the environment, which results in their bioaccumulation and sustainability in food chains, especially fish and aquatic species (Fadaei et al., 2015). Because of their extensive usage from the 1930s to the 1970s, significant levels of PCBs can be found in all components of the global ecosystem. Their contamination can be enhanced during events such as hurricanes, floods, heavy rain, and storms. These events can result in mobilization and redistribution of PCB contaminated sediments, thus enhancing exposures and adverse health impacts in vulnerable populations at the site of disasters. Coplanar PCB congeners such as PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 126 (3,3',4,4',5-pentachlorobiphenyl) are non-ortho substituted and dioxin-like PCBs (Figure 31A and B) (Safe, 1994). Non-coplanar PCB congeners such as PCB 153 (2,2',4,4',5,5'-

*Reprinted with permission from "Strong adsorption of polychlorinated biphenyls by processed montmorillonite clays: potential applications as toxin enterosorbents during disasters and floods" by Wang, M., Safe, S., Hearon, S.E., Phillips, T.D., 2019. *Environmental Pollution*, 255, Copyright 2019 by Elsevier.

hexachlorobiphenyl, di-ortho-substituted) are ortho substituted that do not exhibit dioxin-like toxicities and these compounds are detected at higher levels than the dioxin-like PCBs (Figure 31C). PCB 153 is among the most predominant of the PCBs found in human tissue and is sometimes used as an indicator for the total human PCB-burden (Johansson et al., 2006). To investigate the structural mechanism of PCB adsorption, hexachlorobiphenyl PCBs, other than PCB 153, have also been studied, including PCB 157 (2,3,3',4,4',5'-hexachlorobiphenyl, mono-ortho-substituted), PCB 154 (2,2',4,4',5,6'-hexachlorobiphenyl, tri-ortho-substituted) and PCB 155 (2,2',4,4',6,6'-hexachlorobiphenyl, tetra-ortho-substituted) (Figure 31D-F). These PCB congeners have different toxicological, structural and chemical properties based on variations in the amount and position of chlorine substitutions around the biphenyl rings, and they represent model substances for the different classes of PCBs.

Due to the lipophilic nature of PCBs, they tend to be strongly sorbed by soils and sediments, resulting in widespread PCB contaminated sediments. Studies have reported that activated carbon is the most effective sorbent for PCBs and is used in remediation of PCB contaminated soil, but adsorption isotherms for PCBs by activated carbon in water suggest a weak physisorption activity, although the thermodynamics of this interaction have not been reported (Fairey et al., 2010, McDonough et al., 2008). Additionally, incomplete combustion contributes to the formation of polycyclic aromatic hydrocarbons (PAHs) and other hazardous organic contaminants, which limits the use of carbon as a toxin enterosorbent for human and animal consumption (Mohammad-Khah and Ansari, 2009). Other studies have reported that soil organic matter (SOM) was the major sorptive

compartment in soils and sediments for PCBs, however a partitioning mechanism was also considered as the primary toxin/surface interaction (Hiraizumi et al., 1979, Liu et al., 2015).With physisorption mechanisms, PCBs may be attached to the surface of the adsorbent through weak interactions, with an enthalpy (heat of sorption) less than 20 kJ/mol. Olestra^{TM}, a nonabsorbable fat substitute for potato chips, has been reported to decrease PCBs in rats and humans (Geusau, et al., 1999, Jandacek et al., 2014). However, it was banned from food markets in the U.K. and Canada due to side effects in gastrointestinal track and weight gain and was mandated by FDA to display warning labels (Nestle, 1998, Swithers et al., 2011). Therefore, the objective of this study was to develop a toxin binding clay with high capacity and enthalpy for PCBs that can reduce human and animal exposures when included in food or water. PCB binding enterosorbents can be delivered before each meal in the form of powder, pills, capsules, tablets, sachets, vitamins, snacks, or flavored water to decrease the frequent exposure of humans to PCBs through contaminated fish and seafood.

Previously, montmorillonite clays have been shown to be safe for consumption in animals and humans using multiple animal models and six clinical trials in the US and Africa (Phillips et al., 2019). To develop safe and effective sorbents for PCBs, montmorillonite clays were processed with sulfuric acid (APMs), resulting in the enhancement of active surface area and porosity. The treatment of montmorillonite clays with high concentrations of acid facilitates the replacement of interlayer cations with protons, following the gradual dissociation of tetrahedral and octahedral layers in the clay structure. The final APM product is a mixture of delaminated montmorillonite layers along

with amorphous silica chains, amorphous silica, and cross-linked silica (Figure 31G) (Komadel et al., 1990, Madejova et al., 1998, Tyagi et al., 2006). A pH of 3 in the final product is similar to the pH of acidic foods, such as chocolate, cheese, and meat. In fact, acidic food additives can commonly enhance taste and appetite, and increase palatability and consumption (Deshpande et al., 2015). Additionally, sulfuric acid is permitted in food and feed to adjust pH (Government of Canada, 2017). Thus, APMs for short-term treatment at low inclusion levels in the diet of humans and animals should be safe.

In this study, we have characterized the sorption of six different PCBs onto APMs and investigated thermodynamic and structural mechanisms of sorption. Computational models and isothermal analyses were used to delineate mechanisms and to predict the thermodynamics of sorption. Equilibrium isothermal adsorption and dosimetry studies were conducted to determine sorption parameters and to investigate: 1) sorption capacities and affinities, 2) thermodynamics of toxin/surface interactions, 3) estimated dose of sorbent required to maintain toxin threshold limits, and 4) potential structural mechanisms of sorption. We have also used *Hydra vulgaris* (a living organism) to confirm the safety and predict the efficacy of APMs against individual PCB congeners as well as commercial Aroclors. Aroclors are complex mixtures of PCBs that were extensively used as dielectric fluids in transformers, plasticizers, and heat-exchange fluids. Among these, Aroclors 1254 and 1260 were the most commonly used in the US; they are toxic to laboratory animals (Albro et al., 1981, ATSDR, 2010, EPA, 1989, Mayes et al., 1998), and thus were chosen for studies in the hydra assay.

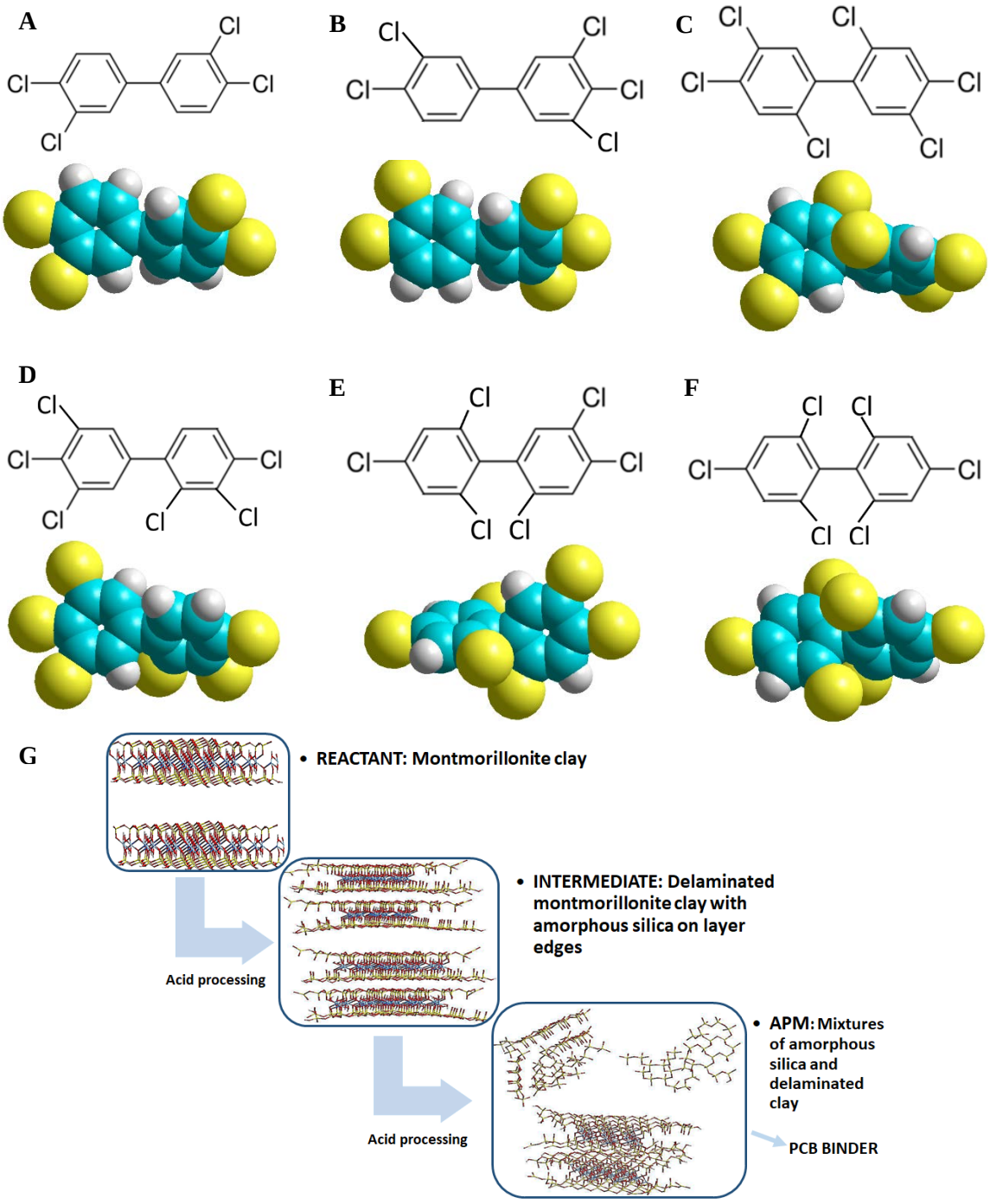

Figure 31. Chemical structures and molecular models of PCB 77 (A), 126 (B), 153 (C), 157 (D), 154 (E), and 155 (F) illustrating spatial orientation and dihedral angles (carbon = cyan; hydrogen = white; chlorine = yellow). Energy minimized molecular models of parent montmorillonite versus APM products (G) (oxygen = red; silicon = yellow; aluminum = cyan).

5.2 Materials and methods

5.2.1 Materials and reagents

Parent montmorillonite used in this study is available from BASF in Lampertheim, Germany with a total surface area of approximately 850 m^2/g and an external surface area of 70 m^2/g (Grant and Phillips, 1998). The generic formula is $(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$. Samples of the clay contain orthoclase feldspars, calcite, mica, quartz, and sanidine as impurities (Phillips et al., 2008). Coconut shell activated carbon was purchased from General Carbon Corporation (Paterson, NJ). This material was selected because it is widely used for the removal of organic compounds and it is suitable for drinking water and food grade applications. Reagents including high pressure liquid chromatography (HPLC) grade acetonitrile and pH buffers were purchased from VWR (Atlanta, GA). Sulfuric acid (H_2SO_4, 95-98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). PCB congeners and Aroclors (purity > 99%) were gifts from Dr. Stephen Safe's laboratory at Texas A&M University (College Station, TX) (Mullin et al., 1981, 1984). Ultrapure deionized water (18.2 MΩ) was generated by an Elga™ automated filtration system in the lab (Woodridge, IL).

Synthesis of APMs was previously described by Wang, et al (2019). Briefly, montmorillonite was treated with sulfuric acid to derive 12 and 18 normality. The clay suspensions were mixed and maintained at 60℃ overnight. The slurry was centrifuged at 2000 g for 20 min and rinsed thoroughly with distilled water until constant pH was reached. All products were desiccated at 110℃ overnight and sieved through 125 μm to achieve the uniform size before use (Neji et al., 2011, Resmi et al., 2012).

5.2.2 Adsorption isotherms

The toxin stock solutions were separately prepared by dissolving pure crystals into acetonitrile to yield 15 ppm (μg/mL) PCB solutions. We used acetonitrile for isothermal analysis because: 1) the solubility of PCBs is limited in water, 2) acetonitrile is a "polar aprotic solvent" that does not affect the interlayer structure of montmorillonite clays like alcohol, and 3) acetonitrile does not interfere with the UV absorbance of PCBs like DMSO. The isotherm methodology was previously described in our laboratory (Grant and Phillips, 1998). Briefly, 0.001% sorbents were added to an increasing gradient of toxin solutions. The toxin gradient was prepared by mixing a calculated amount of toxin stock solution with a complementary volume of acetonitrile. The 0.001% sorbent was obtained by adding 10 μL of 2.0 mg/mL clay suspension with vigorous stirring. Additionally, we tested 3 control groups including acetonitrile, toxin stock solution and 0.001% sorbent in acetonitrile. All groups in disposable glass tubes were capped and agitated at 1000 rpm on shakers for 2 h at ambient temperature (26°C) and body temperature (37°C) for thermodynamic experiments, followed by centrifugation at 2000 g for 20 min to separate the clay/toxin complex from solution. For isothermal analyses, UV-visible spectrophotometry was performed to scan and detect the absorption peaks for PCBs based on previously described methods, where: PCB 77 is detected at 260.9 nm; PCB 126 is detected at 264.5 nm; PCB 153 and 154 are detected at 207.2 nm; PCB 154 is detected at 280 nm; and PCB 157 is detected at 254.9 nm (Andersson et al., 1997, Grant and Phillips, 1998, Wang et al., 2017, 2019).

The highest limit of detection (LOD) was 0.5 ppm for six individual PCB congeners, which was below the concentration gradient of PCBs for isothermal analysis (i.e., 0.75 ppm - 15 ppm). Standard toxin solutions were spiked before and after 2 h of agitating and the relative standard deviations (RSD) were < 4%, indicating a high recovery percentage and limited nonspecific binding. The detection methods were validated using standard calibration curves. Standard solutions of PCBs were prepared in acetonitrile at concentration gradients between 0.25 ppm and 20 ppm to plot the standard curves with high linear correlations ($r^2 > 0.99$).

5.2.3 Data calculations and curve fitting

From the equilibrium isotherms, the toxin concentration in solution (x-axis) was determined using a scanning UV-visible spectrophotometer. The amount of adsorbed toxin at each concentration gradient was calculated from the concentration difference between control and test groups. More specifically, the amount bound (mol/kg) is calculated from the difference between free toxin in the test solution and the control groups divided by the mass of the clay added. The Langmuir equation was used to plot equilibrium isotherms for the toxin/clay binding reactions; the heat of toxin sorption to clay surfaces (ΔH) was derived from the Van't Hoff equation by applying individual K_d values at temperatures of 26°C and 37°C as previously described (Wang et al., 2017, 2019).

5.2.4 Dose of sorbents required to maintain threshold limits of PCBs

In dosimetry studies, 1 ppm PCB test samples were prepared from stock solutions. These solutions were designed to represent twice the threshold limits in drinking water

(i.e., maximum contaminant level = 0.5 ppm) (EPA, 2012). A gradient of sorbent was included to 2.0 mL of toxin solution resulting in 0.005, 0.02, 0.05, 0.1, 0.5 mg sorbent/mL. Control groups included PCB stock solutions. All groups were agitated at 1000 rpm for 2 h and centrifuged at 2000 g for 20 min. Aliquots of toxin were measured by UV-Visible spectroscopy and free toxin concentrations were calculated. The toxin sorption percentage was determined by the difference between test and control groups. Algorithms were derived by methods previously described for other toxins by Wang et al. (2019). This procedure was used in this study to predict therapeutic doses of sorbents for PCBs.

5.2.5 Hydra assay

Hydra vulgaris were obtained from Environment Canada (Montreal) and kept at 18ºC. A morphological method was used to describe adult hydra and classify the adverse effects of toxins (Wilby et al., 1990). In the hydra assay, mature and non-budding hydra of similar size were tested to minimize differences between samples. Individual toxin groups included 40 ppm PCB 77, 30 ppm PCB 126, 30 ppm PCB 153, 20 ppm Aroclor 1260 and 20 ppm Aroclor 1254 in the aqueous hydra media with 1% DMSO based on predetermined toxicity levels at 92 h. The hydra method was used to screen the safety of PCBs and the ability of test sorbents to protect a living organism in aqueous solution. All sorbent/toxin mixtures were shaken at 1000 rpm for 2 h and centrifuged at 2000 g for 20 min, prior to exposure of hydra to toxins in Pyrex dishes. Three adult hydra in each group were added to 4.0 mL of test media and maintained at 18°C. The monitoring times were at shorter intervals during the first two days and 24 h intervals for the last three days (0, 4, 20, 28, 44, 68, and 92 h). The hydra morphological response was scored from 0-10, where

10 indicated healthy hydra and 0 indicated lethality following treatment. Toxicity was calculated from the average score for morphological changes at a specific time point for each group.

5.2.6 Molecular model protocols

The molecular models for PCBs and APMs were drawn in ISIS Draw 2.0 (MDL Information Systems, Inc., Hayward, California) and then imported into HyperChem 8.0. All chemical structures were energy-minimized using the semi empirical quantum mechanical AM1 method. The unit cell coordinates of muscovite were used to construct the models (Richardson and Richardson, 1982). These coordinates were then converted to orthogonal coordinates and the unit cells defining the clay structure were replicated in three-dimensional space using the symmetry operations for a C2/c space group (Donnay, 1952). The d_{001} spacing of the clay model was then set to 21 Å (Greenland and Quirk, 1960). The molecular conformations of PCBs were constructed with the fixed dihedral angles between the biphenyl rings, thus allowing strain energies between adjacent atoms to be minimized.

5.2.7 Statistical analysis

Statistical significance was calculated by a two-way t-test. Each experiment was independently triplicated to derive an average and standard deviation. Morphological scores in the hydra assay were included to calculate difference between media control (score 10) and test groups. The t-value was calculated (N = 3) and compared in a p-value table to determine the statistical significance. Results were considered significant at $p \leq 0.05$.

5.3 Results and discussion

Figure 31 illustrates two common coplanar PCBs, i.e. PCB 77 and 126 (Figure 31A and B), four hexachlorobiphenyl isotherms with variable substitution patterns, i.e. PCB 153, 157, 154, 155 (Figure 31C-F) and energy minimized molecular models of APMs (Figure 31G). The isotherms for the sorption of the most dominant PCB congeners found in the environment onto the surface of parent montmorillonite, APM clays, and activated carbon at 26ºC are shown in Figure 32. The coplanar PCBs (PCB 77 and 126) exhibited a higher binding capacity (Q_{max}) and affinity (K_d) than the non-coplanar PCB 153 in the presence of parent montmorillonite clay or the APMs or activated carbon. The binding of coplanar PCBs fit the Langmuir model for all three types of sorbents, indicating homogeneous and saturable binding sites for these congeners. Isotherms for the non-coplanar PCBs indicated a partitioning interaction of toxins on the surfaces of montmorillonite clay and activated carbon. Similar PCB sorption trends were previously reported for other organic and inorganic sorbents at lower levels of toxin dissolved in aqueous media, and these studies were consistent with our isotherms in acetonitrile. The notable difference in sorption effectiveness between the different PCB congeners can probably be explained by steric effects. The dihedral angles between the biphenyl rings are fixed at the following three values: 44° for PCB congeners without ortho substitution (PCB 77 and 126), 57° for congeners with ortho substitution on one phenyl ring (PCB 157), and 74° for congeners with ortho substitution on both rings (PCB 153, 154 and 155) (Dunnlvant and Elzerman, 1992). This result agrees with previous studies showing that coplanar PCBs showed higher adsorption than non-coplanar PCBs (Liu et al., 2015). Our

results (using acetonitrile as the solvent vehicle) showed that the isotherm plots of APMs with PCB 77, 126 and 153 all fit the Langmuir sorption isotherm model ($r^2 > 0.8$), indicating saturable binding sites for PCBs on APM surfaces. The derived Q_{max} values of APMs increased from the parent montmorillonite and activated carbon for all three PCBs, suggesting improved binding ability of APMs versus parent montmorillonite and carbon; this increase is probably due to 50% higher surface areas (more porosity) and more variations of types of binding sites than that of parent clays and carbon (Wang et al., 2019).

To further investigate the structural mechanism of PCB binding, other hexachlorobiphenyl PCBs were also studied, including PCB 157 (2,3,3',4,4',5'-hexachlorobiphenyl, mono-ortho-substituted), PCB 154 (2,2',4,4',5,6'-hexachlorobiphenyl, tri-ortho-substituted) and PCB 155 (2,2',4,4',6,6'-hexachlorobiphenyl, tetra-ortho-substituted). Although PCB 157 is coplanar, it has mono-ortho-substitution that results in a higher dihedral angle compared to other coplanar PCBs with no ortho substitution (PCB 77 and 126). As illustrated in Figure 33A, isotherms of PCB 157 showed a Freundlich (partitioning) trend onto montmorillonite clay surfaces, indicating less binding sites and tightness compared to the Langmuir trend shown for PCB 77 and 126. APMs increased the binding to a saturable curve that fit to the Langmuir model, but its binding capacity is slightly lower than that of PCB 77 and 126. Like PCB 153, PCB 154 also has ortho-substitution on both rings and this results in similar binding isotherm plots with comparable Q_{max} values (Figure 33B). PCB 155 has four ortho-chlorine substituents on both rings. Although the dihedral angle is fixed and it is the same as PCB 153 and 154, the isothermal results in Figure 33C showed a significantly decreased

Q_{max} for PCB 155, suggesting that other possible binding mechanisms in addition to the dihedral angle are important. It is possible that the number of ortho chlorine substitution can limit the access to the interlayers or pores of the sorbents due to size of the chlorine atoms. For all six PCB congeners tested, APM clays showed the highest binding capacities.

PCBs accumulate in sediments at the bottom of streams, rivers, lakes and coastal areas. These chemicals have also been shown to accumulate in the fatty tissues of fish, shellfish and other animals, and in high concentrations, can pose serious health risks to people who frequently eat contaminated fish and seafood. This problem is magnified during events such as hurricanes, floods, heavy rain, and storms, when PCB contaminated sediments are mobilized and redistributed, thus enhancing exposures and adverse health impacts of these toxin in vulnerable humans and animal populations at the site of disasters. Because fish is an important part of a healthy diet for humans, the ability to limit consumption of PCB-contaminated fish and seafood is highly desirable. Statewide advisories also urge people to limit their consumption of all fish and shellfish from freshwater or coastal areas. For example, Texas Department of State Health Services (DSHS) recently issued a fish possession and consumption ban for various portions of rivers due to high PCB contamination (TCEQ, 2019). Other than seafood, humans are also exposed to PCBs through eating meat and dairy products (Ahmadkhaniha et al., 2017, Chen et al., 2017). PCBs can occur in the fat and viscera of cattle and in milk and eggs. The results of our work suggest that APM could be included in the diet to reduce human and animal exposures to PCBs that are amplified during disasters and flooding. As part of

this study, it was important to gain insight into PCB binding mechanisms onto the surfaces of APMs and the thermodynamics of the toxin/surface interactions. To calculate the binding enthalpy of APM clays for PCBs, isotherms were run at 2 different temperatures, i.e., 26°C (T_1) and 37°C (T_2). Calculated enthalpies (ΔH) for APM-12N and APM-18N were equal to -136 kJ/mol and -86 kJ/mol for PCB 77, -51 kJ/mol and -148 kJ/mol for PCB 126, and -76 kJ/mol and -55 kJ/mol for PCB 153, respectively (Figure 34). These high enthalpy absolute values indicate that the representative coplanar and non-coplanar PCBs are chemisorbed tightly to the APMs, which is consistent with the Langmuir model for the isothermal interaction. A summary of adsorption parameters for six PCB congeners onto surfaces of parent montmorillonite, APMs, and activated carbon is shown in Table 2.

Additionally, we determined a therapeutic dose of APMs required to reduce PCB exposures higher than the action level. The regulatory threshold or maximum contaminant level (MCL) for PCBs in water in the United States is equal to 0.5 ppm. We exposed both coplanar (PCB 77) and non-coplanar (PCB 153) at twice the MCL (1 ppm) to an increasing dose of sorbent treatment from 0.005 g/kg to 0.5 g/kg. An algorithm was derived to calculate the amount of sorbent that would bind 50% of the PCBs, thus maintaining the MCL at 0.5 ppm. The dosimetry results (Figure 35) showed that the predicted inclusion rates for APM-12N and APM-18N were equal to 0.11 g/kg and 0.05 g/kg (w/w) for PCB 77, and 0.01 g/kg and 0.001 g/kg for PCB 153, respectively. Assuming the average adult has a food intake of 3 kg/day and 3 meals/day, then the appropriate dose of APMs could be delivered in a variety of forms, including powder, snacks, capsules, tablets, vitamin supplements, food, and flavored water. Importantly, APMs can be used to protect people

and animals consuming PCB contaminated seafood, meat, dairy products and water at the site of disasters and floods. The low inclusion rates of APMs were consistent with our isothermal results suggesting high capacity binding of PCBs to APM surfaces and confirming that APMs increased binding effectiveness for PCBs more than the parent montmorillonite clay. This data could be helpful to determine dosage requirements for enterosorbent therapy in animals and humans, and other potential applications with APMs during disasters.

The isothermal results, the safety and the efficacy of the APMs were confirmed in a living organism using the hydra assay in aqueous media (Figure 36). Individual toxin exposure with 40 ppm PCB 77, 30 ppm PCB 126 and 30 ppm PCB 153 resulted in severe and irreversible toxicity to hydra. Following the inclusion of 0.1% APMs, adult hydra were completely (100%) protected from PCB 77 toxicity. Also, APM sorbents significantly protected the hydra (*in vivo*) against PCB 126, with protection percentages at the endpoint of the assay equal to 100% and 90% for APM-12N and APM-18N, respectively. At the same inclusion rate, APM sorbents also showed moderate protection against PCB 153 with protection percentages equal to 33% and 50% for APM-12N and APM-18N, respectively. The more significant protection by APMs against PCB 77 and 126 was consistent with the isothermal results indicating that binding was favored for coplanar PCBs more than non-coplanar PCBs. Although there was relatively less protection against PCB 153, the inclusion rate of APM treatment was only 0.1%; the percent protection should be further enhanced with higher inclusion rates of clay based on our previous research.

Aroclor 1254 and 1260 were the most widely used industrial PCBs in the United States and both mixtures are toxic based on laboratory animal studies. As shown in the results of the hydra assay in Figure 37, 20 ppm Aroclor 1260 and 1254 were moderately toxic to hydra, and the toxicity was irreversible. APM treatments at 0.2% inclusion rate were able to provide 80% and 75% protection against individual Aroclor 1260 and 1254, respectively, compared to less from parent montmorillonites (60% and 25% protection). Importantly, this result suggests that APMs can effectively sorb Aroclors and possibly other mixtures of PCBs. Dosimetry studies with Aroclor 1254 and 1260 (data not shown) resulted in averages of 60% and 77% binding of 1 ppm Aroclor 1254 and 1260 at inclusion rates equal to 0.005% and 0.1%, respectively. These *in vitro* results were similar to *in vivo* findings with hydra (Figure 37) and demonstrate the ability of APMs to sorb complex PCB mixtures and reduce their toxicity in a living organism.

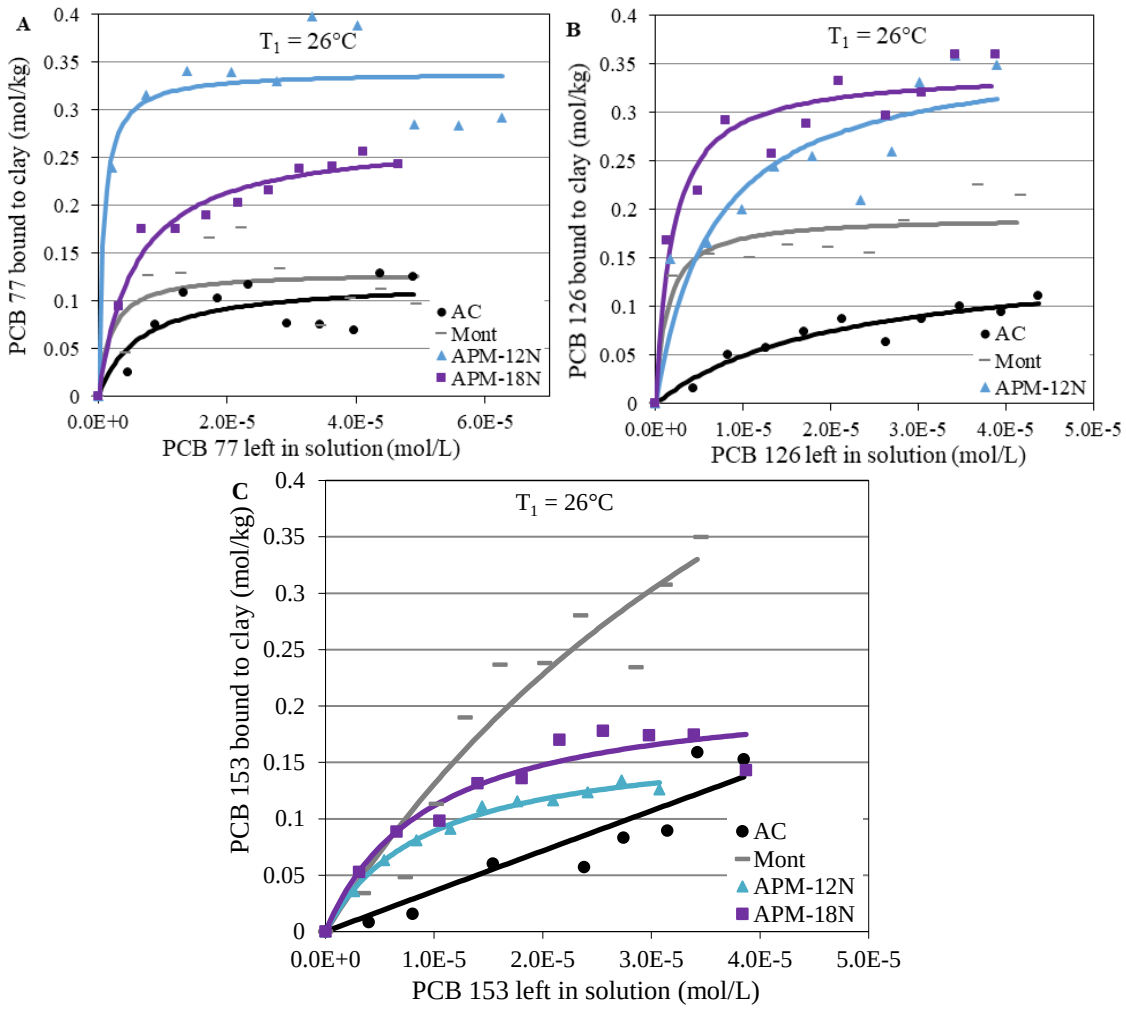

Figure 32. Langmuir plots of coplanar PCB 77 (A) and PCB 126 (B), and non-coplanar PCB 153 (C) bound to the surfaces of APMs versus parent montmorillonite clays (Mont) and activated carbon (AC) at 26°C (T_1).

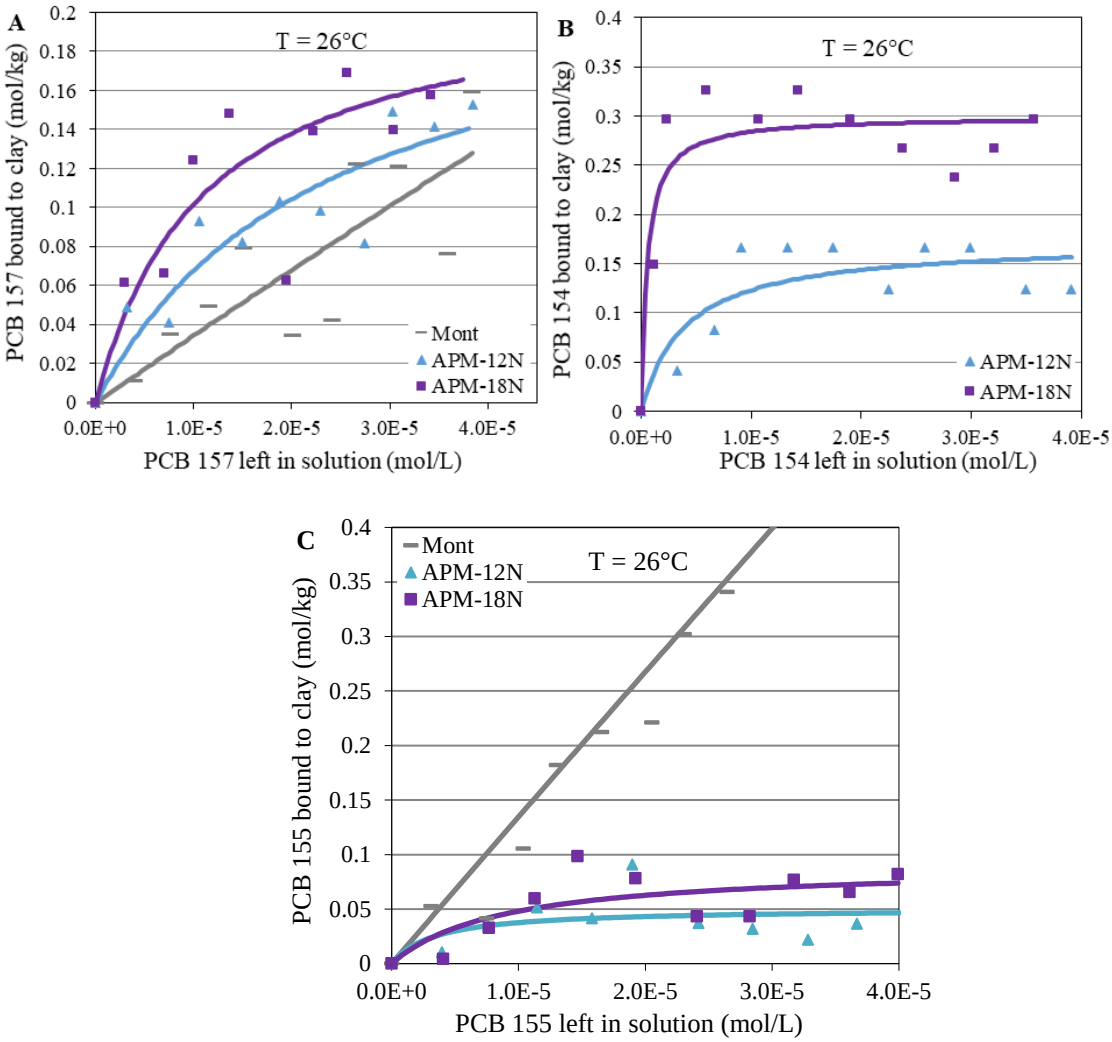

Figure 33. Langmuir plots of hexachlorobiphenyl PCBs, including coplanar PCB 157 (A) and non-coplanar PCB 154 (B) and PCB 155 (C) bound to the surface of APMs versus parent clays (Mont) at 26°C.

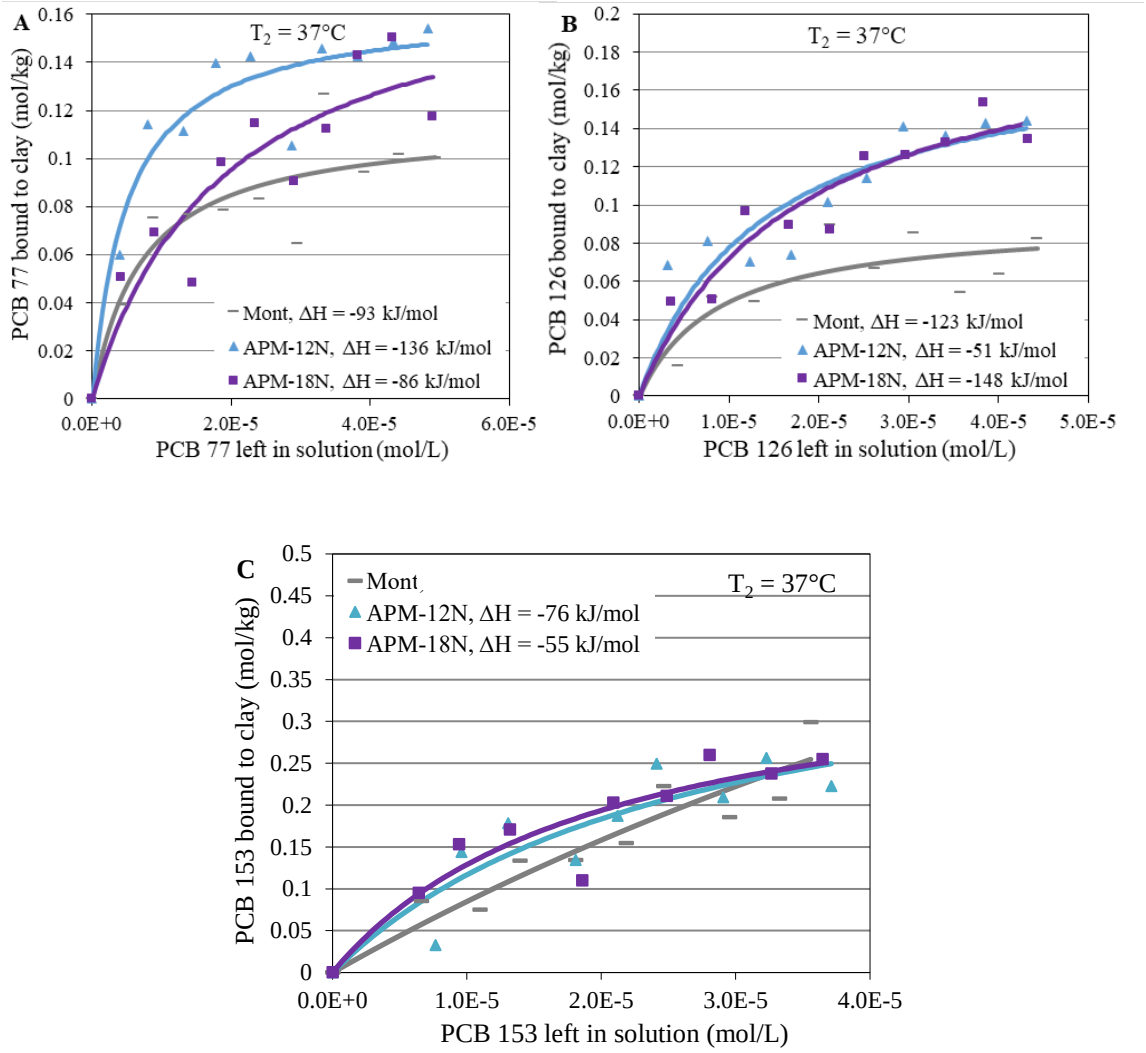

Figure 34. Langmuir plots of coplanar PCB 77 (A) and PCB 126 (B), and non-coplanar PCB 153 (C) bound to the surfaces of APMs versus parent clays (Mont) at 37°C (T_2).

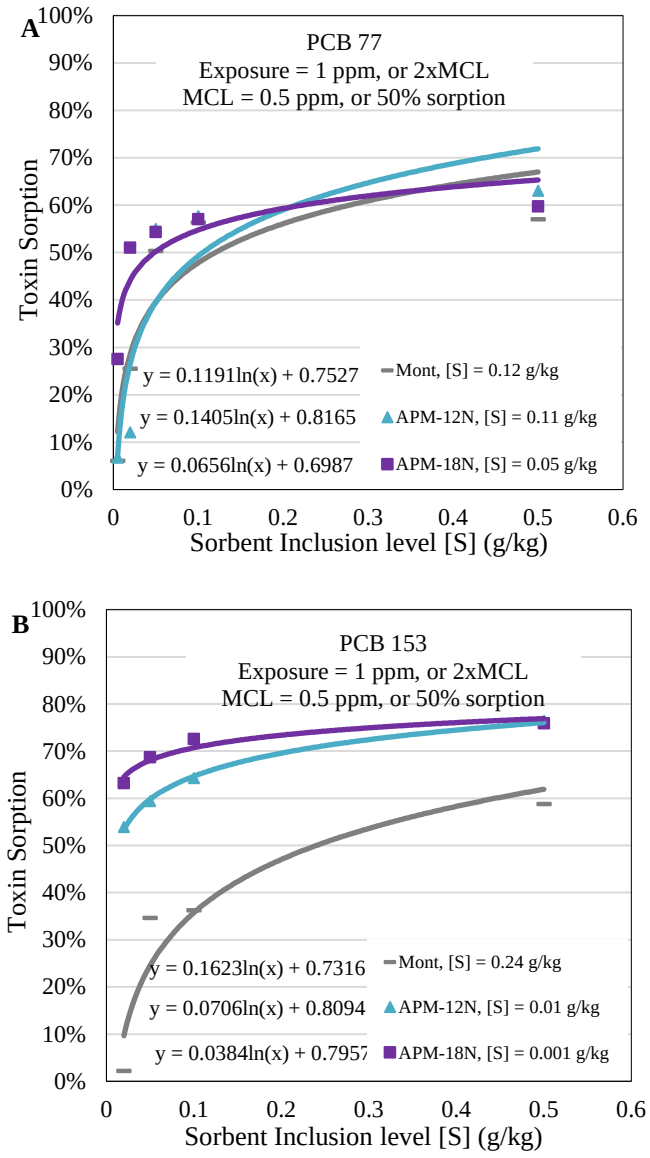

Figure 35. Extrapolation of the sorbent doses required to bind and decrease exposures from 1 ppm PCB 77 (A) and PCB 153 (B).

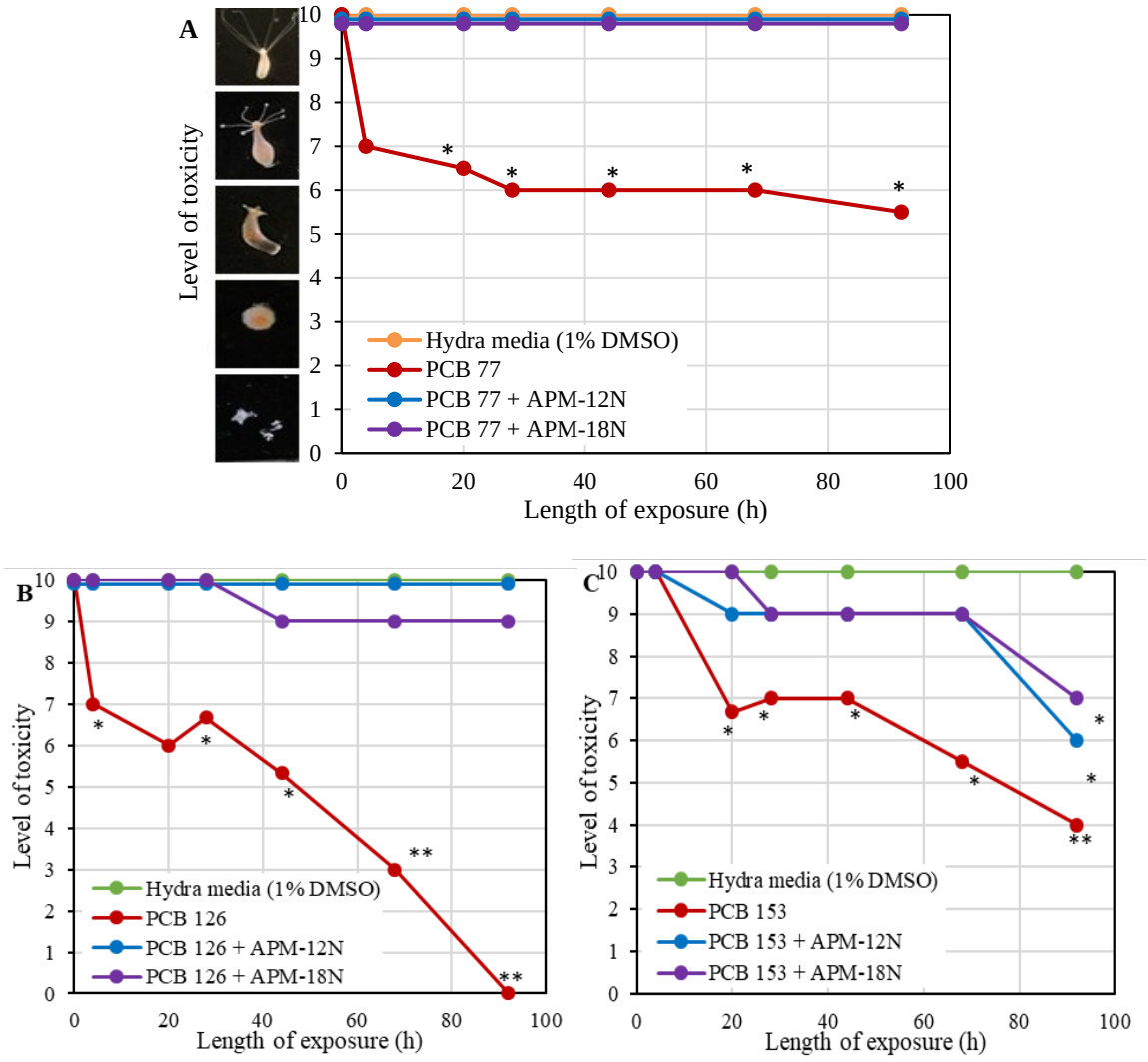

Figure 36. Hydra toxicity and protection by montmorillonite clay and APMs at the 0.1% inclusion level against 40 ppm PCB 77(A), 30 ppm PCB 126 (B), and 30 ppm PCB 153 (C). Hydra media and toxin controls are included for comparison. APM sorbents completely protect (100%) against PCB 77. APM sorbents also protect against PCB 126 and 153. The protection percentages for PCB 126 and 153 at the endpoint are as follows: (B) APM-12N: 100%, APM-18N: 90%. (C) APM-12N: 33%, APM-18N: 50%. (* $p \leq 0.05$, ** $p \leq 0.01$).

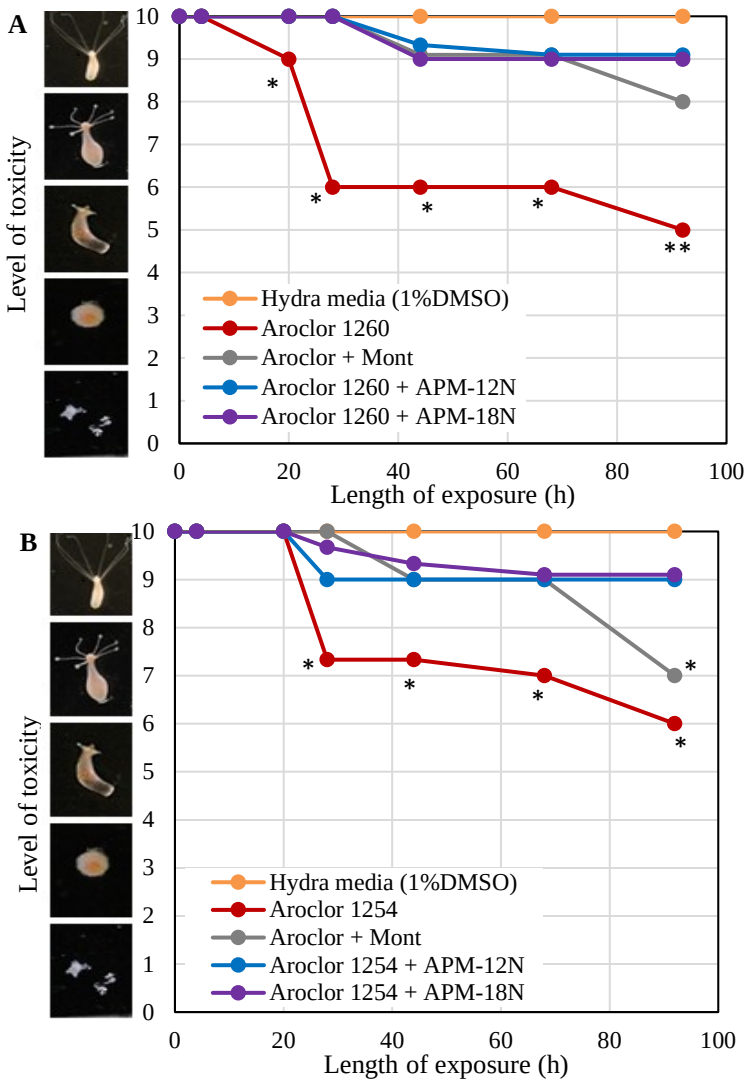

Figure 37. Hydra toxicity and protection by montmorillonite clay and APMs at the 0.1% inclusion level against 20 ppm Aroclor 1260 (A) and 20 ppm Aroclor 1254 (B). Hydra media and toxin controls are included for comparison. APMs showed significant protection against Aroclor toxicity. The protection percentages for Aroclor 1260 and 1254 at the endpoint are as follows: (A) Mont: 60%, APM-12N: 80%, APM-18N: 80%. (B) Mont: 25%, APM-12N: 75%, APM-18N: 75%. (* $p \leq 0.05$, ** $p \leq 0.01$).

Table 2. Summary table of binding parameters for sorbents

	PCB 77 T_1	PCB 77 T_2	PCB 126 T_1	PCB 126 T_2	PCB 153 T_1	PCB 153 T_2	PCB 157 T	PCB 154 T	PCB 155 T
Mont	Q_{max} = 0.13 K_d = 5E5	Q_{max} = 0.11 K_d = 1E5	Q_{max} = 0.19 K_d = 8E5	Q_{max} = 0.09 K_d = 1E5	K_d = 2E5	K_d = 8E4	K_d = 1E3	Q_{max} = 0 K_d = 0	K_d = 6E2
APM-12N	Q_{max} = 0.34 K_d = 1E6	Q_{max} = 0.16 K_d = 2E5	Q_{max} = 0.36 K_d = 2E5	Q_{max} = 0.18 K_d = 7E4	Q_{max} = 0.1 K_d = 1E5	Q_{max} = 0.43 K_d = 4E4	Q_{max} = 0.23 K_d = 4E4	Q_{max} = 0.16 K_d = 3E5	Q_{max} = 0.05 K_d = 3E5
APM-18N	Q_{max} = 0.27 K_d = 2E5	Q_{max} = 0.19 K_d = 5E4	Q_{max} = 0.34 K_d = 5E5	Q_{max} = 0.2 K_d = 6E4	Q_{max} = 0.22 K_d = 1E5	Q_{max} = 0.39 K_d = 5E4	Q_{max} = 0.21 K_d = 9E4	Q_{max} = 0.3 K_d = 2E6	Q_{max} = 0.09 K_d = 1E5
AC	Q_{max} = 0.12 K_d = 2E5	N/A	Q_{max} = 0.15 K_d = 5E4	N/A	K_d = 3E2	N/A	N/A	N/A	N/A

5.4 Conclusion

Based on a combination of *in vitro, in vivo* and molecular simulation studies of APM sorption of coplanar and non-coplanar PCBs, the mechanism appears to involve steric hindrance associated with ortho-chloro substituents, which limit or restrict access of the potent compound to the surfaces and pores of montmorillonite clay and activated carbon, reducing their sorption capacities and affinities. This restriction was overcome by APMs with higher surface area, porosity, and chains of amorphous silica at the edges of the clay that provided increased binding sites, resulting in higher binding capacities, affinities and enthalpies for both coplanar and non-coplanar PCBs. Moreover, APMs maintained threshold limits at low dose levels and resulted in effective protection against PCBs and Aroclors.

Besides PCBs, it is possible that APMs, and similar materials, will be broad-acting to reduce exposures to diverse environmental contaminants in food and drinking water. The short-term administration of APMs in the diet of humans and animals can minimize unintended toxin exposures from contaminated food and water supplies.

6. SUMMARY

Aflatoxins are known human carcinogens that contaminate dietary staples such as corn and peanuts. Aflatoxins have also been demonstrated in animal models to compromise the immune system, interfere with protein metabolism and micronutrients that are critical to health, cause growth stunting and liver cancer; human epidemiological studies have supported these findings. Contamination levels vary with seasons, but are largely driven by drought stress in the field and poor storage conditions post-harvest. Innovative strategies that significantly diminish the bioavailability of aflatoxins and mitigate human and animal exposures from contaminated food and feed have been developed. Extensive previous work based on montmorillonite clays has resulted in the development of a safe and practical therapeutic strategy that can reduce human and animal exposure to aflatoxins, when a broad-acting toxin enterosorbent is delivered by tablet, food, snacks, water and feed. The efficacy of this strategy for aflatoxin adsorption has been confirmed based on high binding capacity, affinity and enthalpy of selected clays; the safety of this strategy has been demonstrated in animal interventions and human clinical trials; and the commercial technology associated with this strategy is being translated globally for mycotoxin mitigation (Figure 38).

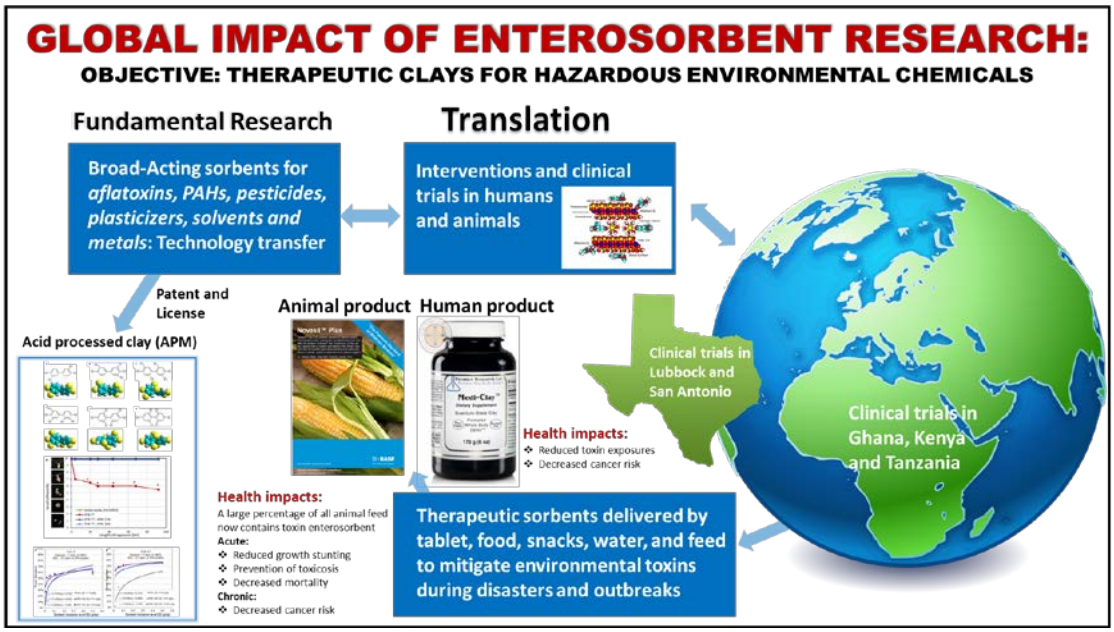

Figure 38. Global impact of toxin enterosorbent work based on fundamental research and translation of clay-based applications.

To further enhance the binding efficacy for aflatoxin, parent sodium and calcium montmorillonites were amended with the natural nutrients L-carnitine and choline. Carnitine and choline are essential nutrients for fat metabolism, serum leptin concentration and energy production. Deficiencies in L-carnitine and choline have been reported to be associated with liver disease and cardiac and muscle metabolic diseases. Carnitine and choline, due to a positive charge from quaternary ammonium groups, were readily exchanged with interlayer cations in the parent montmorillonite clays and the products were not easily dissociated. Modifying calcium and sodium montmorillonites with carnitine and choline reduced the swelling of clay in water, compared to the parent calcium (CM) and sodium (SM) montmorillonites. This resulted from a decreased hydration ability

and decreased H_2O/cation ratio of organic cations (Hensen and Smit, 2002). Importantly, carnitine and choline amended montmorillonites significantly increased their binding capacity and efficacy for aflatoxins, which possibly resulted from 1) increased hydrophobicity of binding sites created by the replaced organic cations, 2) increased d_{001} spacing of the amended montmorillonites, facilitating the access of aflatoxin into the interlayer, and 3) prevention of interlayer collapse, (Jaynes and Zartman, 2011). This modification by L-carnitine and choline provides a novel approach to stabilize swelling sorbents (like SM) and limited swelling sorbents (like CM), and increase the aflatoxin binding capacity versus parent clays. The increased binding from isothermal analyses (*in vitro*) was consistent with *in vivo* protection of adult hydra against AfB_1. These findings suggest that modification of L-carnitine and choline with clay minerals (especially sodium montmorillonites) may be effective as enterosorbent therapy for aflatoxins.

Based on our previous studies, selected montmorillonite clays were effective as aflatoxin enterosorbents for humans and animals with high affinity and capacity, but there are no reports, other than for activated carbon, of similar sorbents for ZEN and other hazardous environmental chemicals. With acid processed montmorillonites (APMs), we have simulated the structure of activated carbon with high surface area and porosity. This is important because incomplete combustion for the process of activated carbon contributes to the formation of polycyclic aromatic hydrocarbons (PAHs) and other hazardous organic contaminants, which limits the use of carbon as a toxin enterosorbent for human and animal consumption (Mohammad-Khah and Ansari, 2009). More importantly, montmorillonite clays are the only toxin sorbents that have been shown to be

safe for human and animal consumption based on numerous published animal studies and six human clinical trials in Africa and the United States. Following our characterization of the physio-chemical properties of APMs, acid treatment was shown to increase clay resistance to high temperatures, decrease its expansibility in water, increase its surface area, decrease its trace metal concentrations and enhance the porosity of the final product. These changes were derived from the extraction of inorganic cations from the interlayer surfaces and basal clay structures, and the replacement and addition of excessive protons on the clay surfaces during the acid treatment.

As indicated in Chapter 4, acid treated calcium-rich montmorillonite (APCM) and sodium-rich montmorillonite (APSM) clays were able to maintain the adsorption of aflatoxin with similar binding curves and capacities. More importantly, acid treatment significantly improved ZEN binding onto the surface of CM and SM, from a nonspecific Freundlich plot, to a saturable Langmuir plot indicating a saturable site and potential tight binding. The high binding capacity of ZEN ($Q_{max} > 0.2$) confirmed the ability of APMs to serve as effective ZEN enterosorbents. From the scientific literature, the parent clays do not significantly bind ZEN and are not effective as enterosorbents. The mechanism of ZEN binding to APMs may be related to the higher surface areas and mesoporosities of APMs versus parent clays. The tight binding also is reflected by the high enthalpies of sorption ($\Delta H < -70$ kJ/mol), which indicates that the interaction energy for the ZEN binding was almost 4 times more than a weak attraction (or physisorption). This is the first report of a clay-based sorbent that is an effective binder of ZEN, with high binding capacity and enthalpy. It also should be relatively safe for short-term human and animal

consumption, based on extensive studies with the same parent clays in animals and humans, along with our hydra studies that show that APMs are not toxic at levels that can protect the hydra from ZEN. Aflatoxins were shown to bind mainly within APM interlayers, whereas, ZEN favored the more organophilic basal surfaces and edge sites of APMs. These differences between binding sites and mechanisms of toxin sorption contribute to the ability of APMs to adsorb a mixture of both mycotoxins at the same time, with limited interference. The *in vivo* hydra assay further confirmed the safety and efficiency of APMs against individual mycotoxins, and common mycotoxin mixtures of aflatoxin and ZEN.

The translation of enterosorbent therapy for aflatoxins resulted in a spinoff study to investigate broad-acting, field-practical and cost-effective sorbents for hazardous environmental chemicals. This strategy is important during natural and man-made disasters (such as hurricanes and flooding), when chemical contaminants can be mobilized and redistributed in the environment, exposing humans and animals to contaminated soil and sediment, and threatening the safety of municipal drinking water and critical food sources (Figure 39).

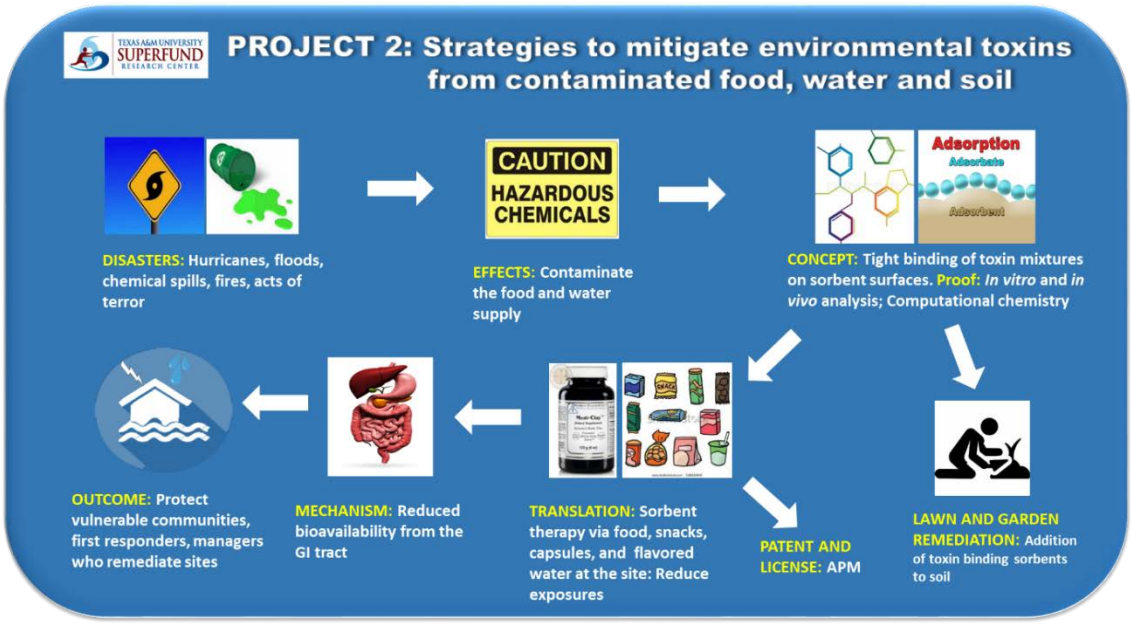

Figure 39. Enterosorbent strategy to mitigate environmental toxins from contaminated food, water and soil outlined in project 2 of our Superfund grant.

To investigate the broad-acting sorbents for the detoxification of hazardous environmental contaminants, and to gain insight into sorption mechanisms, binding parameters and physio-chemical properties of contaminants, both *in vitro* and *in vivo* studies were conducted with multiple classes of organic chemicals such as industrial solvents, PAHs, pesticides, PCBs and plasticizers that have been prioritized by ATSDR as important hazardous substances. The environmental chemicals tested in this study are summarized in Table 3. For each individual chemical, binding efficacy was shown by the intensity of color (0-100%) for each binding parameter (Table 4). These intensities were standardized based on results from previous *in vitro* and *in vivo* work with aflatoxin. The efficacy of sorption of chemicals, representing diverse chemical classes, onto the surfaces

of clays and activated carbons is presented by color (and intensity of color) within the rectangles in Table 5. The color intensity displayed for each class of chemical represents the average binding efficacy of individual chemicals in that class. Compared to parent clays, processed and amended sorbents significantly enhanced the efficacy of all 5 classes of chemicals, especially industrial solvents, pesticides and PCBs, in terms of high binding capacity and affinity, chemisorption, low therapeutic dose and effective protection *in vivo*. Activated carbons derived from coconut shell and hardwood were shown to be the best sorbents for PAHs and plasticizers. Thus, it is possible that clay-based therapy for combinations of these chemicals may consist of a sorbent mixture, where the composition can be adjusted based on the presence and concentration of individual chemical contaminants.

Table 3. Prioritized environmental contaminants tested in project 2

Substance	CAS	Substance	CAS
Phenol	108-95-2	Dieldrin	60-57-1
Benzene	71-43-2	Atrazine	1912-24-9
Toluene	108-88-3	2,4-D	94-75-7
Xylene (3)	1330-20-7	Trifluralin	1582-09-8
PCBs (6) & Aroclors (2)	N/A	Dinitrophenol	51-28-5
Benzo[a]pyrene	50-32-8	Paraquat	4685-14-7
Pyrene	129-00-0	Pentachlorophenol	87-86-5
Naphthalene	91-20-3	Trichlorophenol	88-06-2
Benzo[b]fluoranthene	205-99-2	p, p'-DDT	50-29-3
Lindane	58-89-9	Bisphenol A	80-05-7
Diazinon	333-41-5	Bisphenol S	80-09-1
Aldicarb	116-06-3	Bisphenol F	620-92-8
Linuron	330-55-2	Di(2-ethylhexyl) phthalate	117-81-7
Glyphosate	1071-86-3	Di-*n*-butyl phthalate	84-74-2
AMPA	74341-63-2	Chlorpyrifos	2921-88-2

Table 4. Intensity of binding (color key)

Binding Parameters	100%	50%	0%
Binding capacity	$Q_{max} \geq 0.3$ mol/kg	$Q_{max} = 0.15$ mol/kg	$Q_{max} = 0$ mol/kg or N/A
Binding affinity	$K_d \geq 10^5$	$10^5 > K_d > 10^4$	$K_d \leq 10^4$
Binding enthalpy	$\Delta H > -20$ kJ/mol (chemisorption)	N/A	$\Delta H \leq -20$ kJ/mol (physisorption)
Therapeutic dose	MED ≤ 2 g/kg	N/A	MED > 2 g/kg
Protection *in vivo*	100% protection at 0.1% inclusion	50% protection at 0.1% inclusion	no protection at 0.1% inclusion

(N/A: not applicable; MED: minimum effective dose)

Table 5. Color array depicting the average binding efficacy of representative classes of environmental contaminants listed in Table 3

Superfund chemical classes	Activated carbon: coconut and hardwood	Parent montmorillonite clays	Processed montmorillonite clays
PAHs (4)			
Plasticizers (5)			
Solvents (6)			
Pesticides (16)			
PCBs (8)			

(Purple = high binding capacity; Red = high binding affinity; Green = chemisorption; Blue = low therapeutic dose; Brown = protection *in vivo*)

Adsorption studies with prioritized chemicals including BaP, aldicarb, glyphosate and PCBs were used for the development of broad-acting enterosorbents. They were selected based on their acute toxicities, wide distribution and importance regarding human and animal exposures.

BaP is a well-known environmental pollutant and a human and animal carcinogen that is commonly found in contaminated water and sediment after natural disasters. EPA reported that BaP was detected in water at high contamination levels that exceed EPA's excess lifetime cancer risk range after Hurricane Katrina in 2005 and Hurricane Harvey in 2017. Besides natural disasters, significant amounts of BaP were found in the dust and smoke after the World Trade Center disaster in downtown New York City. Aldicarb is one of the major pesticides found in water and sediment samples in the US and Canada, including groundwater and drinking water in New York and Wisconsin. Importantly, 50% of the concentration in New York groundwater was above the state standard of 7 ppb; 0.9% of samples contained aldicarb at concentrations above 100 ppb. Glyphosate is one of the most used herbicides to control weeds. Almost 90% of all transgenic crops grown worldwide are glyphosate resistant, and the adoption of these crops is increasing at a steady pace. PCBs accumulate in the sediments at the bottoms of streams, rivers, lakes and coastal areas. These chemicals can build up in the fatty tissues of fish and other animals, and in high concentrations, can pose serious health risks to people who frequently eat contaminated fish.

Isothermal analyses, along with the *in vivo* hydra assay, showed that sorbents, including APMs and carnitine and choline amended clays, very effectively bound these

prioritized chemicals. As shown in Table 6, which summarizes the binding parameters of these chemicals, the parent montmorillonite clays showed no saturable binding, or limited binding with low binding capacities for the four selected chemicals. Whereas, processed and amended montmorillonite clays showed significantly increased binding for the four chemicals, including high binding capacity and affinity, tight binding with high enthalpy, low MED values and high protection percentage *in vivo*. Especially APMs were shown to be optimal sorbents for highly lipophilic compounds such as PCBs and BaP, with the highest binding capacities versus all other clay materials and activated carbons. This is possibly due to the enhanced variety of pore sizes and active binding sites including a mixture of amorphous silicate, amorphous chains at the end of clay layers, protonated clay layers and intact clay layers. This is the first report of a sorbent material (other than activated carbon) with high binding efficacy for these environmental chemicals. The development of APMs and claims for enhanced and broad-acting ability of these materials to reduce environmental contaminant exposures in humans and animals, has recently resulted in a provisional patent (62/719,924) and a non-provisional patent application (under review) describing this discovery. Also, a worldwide exclusive license has been granted to TESI, Inc. in September, 2018 through Texas A&M University. It will be possible (following animal safety studies) that small capsules or tablets, snacks, vitamin supplements, and flavored water containing these newly developed sorbents can be administered to humans and animals before each meal to significantly reduce toxin exposures during a disaster. This therapy could be used in vulnerable communities, first

responders and remediation personnel at the site of disasters, such as hurricanes, floods, fires, acts of terror, and droughts to reduce the impact of toxin exposures.

Activated carbons have shown a significant sorptive effect on many of the chemicals tested, with non-specific binding, and most of these are readily available to the consumer. Thus, activated carbon plus clays, at very low inclusion rates, could enhance the broad-acting effect of the toxin enterosorbent strategy. Moreover, the clay components in the sorbent mixture, such as APMs and amended clays, can be adjusted based on the individual contaminants, and concentrations, if known.

Table 6. Comparison of binding parameters of sorbents for prioritized environmental contaminants

	Glyphosate	PCBs (PCB77)	BaP	Aldicarb
Activated Carbon	Q_{max} = 0.47 mol/kg K_d = 3.1E5	Q_{max} = 0.12 mol/kg K_d = 2E5	Q_{max} = 0.1 mol/kg K_d = 2.1E5 MED = 3.4 g/kg 33% *in vivo* protection	Q_{max} = 0.98 mol/kg K_d = 3.1E5 0% *in vivo* protection
Parent montmorillonite	Q_{max} = 0.34 mol/kg K_d = 2.4E5 ΔH = -36 kJ/mol MED = 3.6 g/kg 60% *in vivo* protection	Q_{max} = 0.13 mol/kg K_d = 5E5 ΔH = -93 kJ/mol MED = 0.12 g/kg 100% *in vivo* protection	N/A Q_{max} K_d = 3E3	N/A Q_{max} K_d = 8.9E2
APM-12N	Q_{max} = 0.42 mol/kg K_d = 1.8E5 ΔH = -37 kJ/mol MED = 2 g/kg 100% *in vivo* protection	Q_{max} = 0.34 mol/kg K_d = 1E6 ΔH = -136 kJ/mol MED = 0.11 g/kg 100% *in vivo* protection	Q_{max} = 0.16 mol/kg K_d = 8.6E5 50% *in vivo* protection	Q_{max} = 0.4 mol/kg K_d = 4.3E5 50% *in vivo* protection

Table 6. Continued

	Glyphosate	PCBs (PCB77)	BaP	Aldicarb
APM-18N	Q_{max} = 0.58 mol/kg K_d = 1.3E5 ΔH = -22 kJ/mol MED = 1.38 g/kg 100% *in vivo* protection	Q_{max} = 0.27 mol/kg K_d = 2E5 ΔH = -86 kJ/mol MED = 0.05 g/kg 100% *in vivo* protection	Q_{max} = 0.23 mol/kg K_d = 2.4E6 50% *in vivo* protection	Q_{max} = 0.48 mol/kg K_d = 3E6 50% *in vivo* protection
Mont-carnitine	Q_{max} = 0.4 mol/kg K_d = 1.6E6 ΔH = -152 kJ/mol MED = 2.8 g/kg 100% *in vivo* protection	Q_{max} = 0.22 mol/kg K_d = 1E6	Q_{max} = 0.09 mol/kg K_d = 8.5E4 ΔH = -42 kJ/mol 67% *in vivo* protection	Q_{max} = 0.55 mol/kg K_d = 1.8E6 100% *in vivo* protection

Table 6. Continued

	Glyphosate	PCBs (PCB77)	BaP	Aldicarb
Mont-choline	Q_{max} = 0.42 mol/kg K_d = 3.9E5 ΔH = -128 kJ/mol MED = 3.8 g/kg 100% *in vivo* protection	Q_{max} = 0.09 mol/kg K_d = 4E5	Q_{max} = 0.09 mol/kg K_d = 9.0E4 ΔH = -46 kJ/mol 67% *in vivo* protection	Q_{max} = 0.53 mol/kg K_d = 1.9E6 100% *in vivo* protection

(Q_{max}: binding capacity in mol/kg; K_d: binding affinity; ΔH: binding enthalpy in kJ/mol; MED: minimum effective dose in g of sorbent/kg of daily intake; N/A: not applicable; *In vivo* assay was conducted with 0.1% sorbent inclusion and toxin concentration causing 100% mortality in hydra)

In summary, the novelty of this study is to develop therapies to minimize unintended exposures of humans and animals to food-borne mycotoxins and environmental chemicals in contaminated drinking water and food. This is especially important during natural disasters such as droughts and flooding, since most strategies following disaster emergencies focus on environmental remediation. In a ground-breaking approach, we have developed broad-acting sorbents (including acid processed montmorillonites (APMs) and montmorillonites amended with carnitine and choline) that are "generally recognized as safe" for short-term consumption. The sorption efficacy of the developed sorbents was characterized for diverse classes of chemicals including mycotoxins, pesticides, plasticizers, industrial solvents, PAHs and PCBs. For each chemical and sorbent, the following criteria were determined: binding efficacy including binding capacity, affinity and enthalpy; minimum effective dose (MED) that will meet the regulatory level of toxins; and ability of the sorbent to protect a living organism. We have also used molecular simulations and computational chemistry to study sorption mechanisms and confirm our *in vitro* and *in vivo* results. In this dissertation, diverse chemicals such as aflatoxin, zearalenone, glyphosate, aldicarb, benzo[a]pyrene, PCBs and Aroclors were shown to bind to novel developed sorbents, including carnitine/choline amended clays and APMs. Importantly, the development of the APMs resulted in a provisional patent, a non-provisional patent application, and a worldwide exclusive license granted through Texas A&M University. Possibly, the ultimate product from these studies will involve sorbent mixtures that can be adjusted (or tuned) based on the chemical concentrations at contaminated sites or spills and emergencies. These novel sorbents can

be delivered to animals and humans during disasters to tightly bind and detoxify hazardous environmental toxins in the stomach and intestines, and thus decrease bioavailability and toxicity.

REFERENCES

Abbes, S., Ouanes, Z., Ben Salah-Abbes, J., Houas, Z., Oueslati, R., et al. (2006a). The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. Toxicon. 47, 567-574.

Abbes, S., Salah-Abbes, J.B., Ouanes, Z., Houas, Z., Othman, O., et al. (2006b). Preventive role of phyllosilicate clay on the immunological and biochemical toxicity of zearalenone in Balb/c mice. Int Immunopharmacol. 6, 1251-1258.

Abid-Essefi, S. (2004). Cytotoxicity, inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. Toxicol In Vitro, 18, 467-474.

Abid-Essefi, S., Baudrimont, I., Hassen, W., Ouanes, Z., Mobio, T.A., et al. (2003). DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: prevention by Vitamin E. Toxicol. 192(2-3), 237-248.

Agency for Toxic Substances and Disease Registry. (2015). Priority list of hazardous substances that will be candidates for toxicological profiles. Atlanta, GA: U.S. Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. (2000). Public health statement polychlorinated biphenyls (PCBs). Atlanta, GA: U.S. Department of Health and Human Services.

Ahamed, S., Foster, J.S., Bukovsky, A., Wimalasena, J. (2001). Signal transduction through the ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in MCF-7 cells. Mol Carcinog. 30, 88-98.

Ahmadkhaniha, R., Nodehi, R.N., Rastkari, N., Aghamirloo, H.M. (2017). Polychlorinated biphenyls (PCBs) residues in commercial pasteurized cows' milk in Tehran, Iran. J Environ Health Sci Eng. 15(15).

Akar, S.T., Uysal, R. (2010). Untreated clay with high adsorption capacity for effective removal of C.I. Acid Red 88 from aqueous solutions: Batch and dynamic flow mode studies. J Chem Eng. 162(2), 591-598.

Ake, C.L., Mayura, K., Huebner, H., Bratton, G.R., Phillips, T.D. (2001). Development of porous clay-based composites for the sorption of lead from water. J Toxicol Environ Health A. 63(6), 459-475. DOI: 10.1080/152873901300343489.

Albro, P.W., Corbett, J.T., Schroeder, J.L. (1981). Quantitative characterization of polychlorinated biphenyl mixtures (aroclors® 1248, 1254 and 1260) by gas chromatograpy using capillary columns. J Chromatography A. 205 (1), 103-111.

Al-Degs, Y.S., El-Barghouthi, M.I., El-Sheikh, A.H., Walker, G.M. (2008). Effect of solution pH, ionic strength, and temperature on adsorption behavior of reactive dyes on activated carbon. Dyes Pigments. 77, 16-23.

Ali, M., Mohiuddin, S., Reddy, M. (1994). Effect of dietary aflatoxin on cell-mediated-immunity and serum-proteins in broiler chicken. Indian J Vet. 71(8), 760-762.

Alther, G.R. (1995). Orgnically modiffied clay removes oil from water. Waste Management. 15, 623-628.

Amar, P.K. (2010). Ensuring safe water in post-chemical, biological, radiological and nuclear emergencies. J Pharm Bioallied Sci. 2(3), 253-66. doi: 10.4103/0975-7406.68508.

Amari, A., Chlendi, M., Gannouni, A., Bellagi, A. (2010). Experimental and theoretical studies of VOC adsorption on acid-activated bentonite in a fixed-bed adsorber. Ind Eng Chem Res. 49, 11587-11593.

Amari, A., Gannouni, H., Khan, M.I., Almesfer, M.K., Elkhaleefa, A.M., Gannouni, A. (2018). Effect of structure and chemical activation on the adsorption properties of green clay minerals for the removal of cationic dye. Appl Sci. 8, 2302.

Andersson, P.L., Haglund, P., Tysklind, M. (1997). Ultraviolet absorption spectra of all 209 polychlorinated biphenyls evaluated by principal component analysis. Fresenius J. Anal. Chem. 357(8), 1088-1092.

Apul, O.G., Wang, Q., Zhou, Y., Karanfil, T. (2013). Adsorption of aromatic organic contaminants by graphene nanosheets: Comparison with carbon nanotubes and activated carbon. Water Res. 47, 1648-1654.

Associated Press News Website. Hurricane Harvey's toxic impact deeper than public told. https://apnews.com/e0ceae76d5894734b0041210a902218d.

Avantaggiato, G., Greco, D., Damascelli, A., Solfrizzo, M., Visconti, A. (2014). Assessment of multi-mycotoxin adsorption efficacy of grape pomace. J Agri Food Chem. 62, 497-507.

Awuor, A.O., Yard, E., Daniel, J.H., Martin, C., Bii, C., et al. (2017). Evaluation of the efficacy, acceptability and palatability of calcium montmorillonite clay used to reduce aflatoxin B1 dietary exposure in a crossover study in Kenya. Food Addit Contam A. 34, 93-102.

Ayranci, E., Hoda, N. (2005). Adsorption kinetics and isotherms of pesticides onto activated carbon-cloth. Chemosphere. 60(11), 1600-1607.

Azziz-Baumgartner, E., Lindblade, K., Gieseker, K., Rogers, H. S., Kieszak, S., et al. (2005). Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ Health Perspect. 113(12), 1779-1783.

Baumard, P., Budzinski, H., Garrigues, P., Dizer, H., Hansen, P.D. (1999). Polycyclic aromatic hydrocarbons in recent sediments and mussels (Mytilus edulis) from the western Baltic Sea: occurrence, bioavailability and seasonal variations. Mar Environ Res. 47, 17-47.

Beak, D.G., Basta, N.T., Scheckel, K.G., Traina, S.J. (2006). Bioaccessibility of arsenic(V) bound to ferrihydrite using a simulated gastrointestinal system. Environ Sci Technol. 40, 1364–1370.

Berge, W., Mundt, K., Luu, H., Boffetta, P. (2017). Genital use of talc and risk of ovarian cancer: a meta-analysis. Eur J Cancer Prev. 27 (3), 248-257.

Bertrand, N., Bertrand, D. (1991). Photodegradation of the carbamate pesticides aldicarb, carbaryl and carbofuran in water. Anal Chem Acta. 254, 235-244.

Bi, H., Xie, X., Yin, K., Zhou, Y., Wan, S., et al. (2012). Spongy graphene as a highly efficient and recyclable sorbent for oils and organic solvents. Adv Funct Mater. 22, 4421-4425.

Bingham, A.K., Huebner, H.J., Phillips, T.D., Bauer, J.E. (2004). Identification and reduction of urinary aflatoxin metabolites in dogs. Food Chem Toxicol. 42, 1851-1858.

Blum, K., Manzo, L., Eds. Neurotoxicology. Marcel Dekker, Inc., New York, 1985, 406.

Bohm, J. (1992). The significance of the mycotoxins desoxynivalenol, zearalenone and ochratoxin A for agricultural domestic animals. Arch Tierernahr. 42, 95-111.

Bondy, G.S., Pestka, J.J. (2000). Immunomodulation by fungal toxins. J Toxicol Environ Health B, Critical Reviews. 3(2), 109-143.

Bonner, C.M., DeBrie, K.L., Hug, G., Landrigan, E., Taylor, B.J. (1995). Effects of parenteral L-carnitine supplementation on fat metabolism and nutrition in premature neonates. J Pediatr. 126, 287-292.

Borggaard, O.K. Gimsing, A.L. (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci. 64(4), 441-56.

Boström, C.E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., et al. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air [Review]. Environ Health Perspect. 110 Suppl 3, 451-488.

Bowman, J.C., Zhou, J.L., Readman, J.W. (2002). Sorption and desorption of benzo(a)pyrene in aquatic systems. J Environ Monit. 4(5), 761-766.

Boyd, S.A., Mortland, M.M., Chiou, C.T. (1988). Sorption characteristics of organic compounds on hexadecyltrimethylammonium-smectite. Soil Science Society of America 52, 652-657.

Brandli, R.C., Hartnik, T., Henriksen, T., Cornelissen, G., (2008). Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil. Chemosphere. 73(11), 1805-1810.

Brass, E.P., Hiatt, W. R. (1994). Minireview: carnitine metabolism during exercise. Life Sci. 54,1383-1393.

Bremer, J. (1997). The role of carnitine in cell metabolism. De Simone, C. Famularo, G. eds. Carnitine Today ,1-37 Landes Bioscience Austin, TX.

Brightsmith, D.J., Taylor, J., Phillips, T.D. (2008). The roles of soil characteristics and toxin adsorption in avian geophagy. Biotropica. 40(6), 766-774.

Brooks, G.T. (1969). The metabolism of diene-organochlorine (cyclodiene) insecticides, Residue Rev. 27, 81.

Brown, J.F., Wagner, R.E., Dedard, D.L., Brennan, M.J., Carnaban, J.C., May, R.J. Tofflemire, T.J. (1985). PCB dechlorination in upper Hudson sediments, Proc Am Chem Sot. ENVR-0012.

Brown, K.A., Mays, T., Romoser, A., Marroquin-Cardona, A., Mitchell, N.J., Elmore, S.E., Phillips, T.D. (2014). Modified hydra bioassay to evaluate the toxicity of multiple mycotoxins and predict the detoxification efficacy of a clay-based sorbent. J Appl Toxicol. 34(1), 40-48.

Bueno, D.J., Di Marco, L., Oliver, G., Bardon, A. (2005). In vitro binding of zearalenone to different adsorbents. J Foot Prot. 68, 613-615.

Burchiel, S.W., Luster, M.I. (2001). Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. Clin Immunol. 98, 2-10.

Buyukuysal, R.L., Ulus, I.H., Aydin, S., Kiran, B.K. (1995). 3,4-Diaminepyridine and choline increase in vivo acetylcholine release in rat striatum. Eur J Pharmacol. 281,179-185.

Callahan, G.N. (2003). Eating dirt. Emerg Infect Dis. 9(8), 1016-1021.

Carey, J.H., Lawrence, J., Toxine, H.M. (1976). Photodechlorination of PCB's in the presence of titanium dioxide in aqueous suspensions. Bull Environ Contam Toxicol. 1976, 16(6), 697-701.

Celis, R., Trigo, C., Facenda, G., Hermosin Mdel, C., Cornejo, J. (2007). Selective modification of clay minerals for the adsorption of herbicides widely used in olive groves. J Agric Food Chem. 55(16), 6650-6658.

Center for Disease Control. (1979). Epidemiologic notes and reports: Suspected carbamate intoxications-Nebraska. Morbid. Mortal. Week. Rep. 28, 133-134.

Chen, X., Lin, Y., Dang, K., Puschner, B. (2017). Quantification of polychlorinated biphenyls and polybrominated diphenyl ethers in commercial cows' milk from California by gas chromatography–triple quadruple mass spectrometry. PloS One, 12(1), e0170129.

Chen, Y., Abbate, M., Tang, L., Cai, G., Gong, Z., et al. (2014). L-Carnitine supplementation for adults with end-stage kidney disease requiring maintenance hemodialysis: a systematic review and meta-analysis. Am J Clin Nutr. 99, 408-422.

Clayton, G.D., Glayton, F.E. (1981). Halogenated cyclic hydrocarbons, in: Patty's Industrial Hygiene and Toxicology, Vol. 2B, 3645-3669, George and Florence Clayton (Eds.), New York.

Conlay, L.A., Sabounjian, L.A., Wurtman, R.J. (1992). Exercise and neuromodulators: choline and acetylcholine in marathon runners. Int J Sports. 13, 141-142.

Cortés-Arriagada, D. (2017). Adsorption of polycyclic aromatic hydrocarbons onto graphyne: Comparisons with graphene. Int J Quantum Chem. 117, e25346.

Cortinovis, C., Pizzo, F., Spicer, L.J., Caloni, F. (2013). Fusarium mycotoxins: effects on reproductive function in domestic animals–a review. Theriogenology. 80, 557-564. doi: 10.1016/j.theriogenology.2013.06.018

Cotty, P. J. (1991). Effect of harvest date of aflatoxin contamination of cottonseed. Plant Dis. 75(3), 312-314.

Cotty, P.J., Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int J Food Microbiol. 119(1-2), 109-115.

Craddock, J.H. (1982). What we know about health effects of PCBs. Presentation at the Summer National AIChE Meeting, St. Louis, MO, Cleveland, Ohio.

Creppy, E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology letters. 127(1), 19-28.

D’Mello, J.P.F., Placinta, C.M., Macdonald, A.M.C. (1999). Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol. 80, 183-205.

De, B.K., Patel, J.D., Patel, J.B., Patel, V.K., Patel, V.R. (2009). Bleaching of mustard oil with some alternative bleaching agents and acid activated clay. J Oleo Sci. 58, 57-63.

Deshpande, S.A., Yamada, R., Mak, C.M., Hunter, B., Obando, A.S., et al. (2015). Acidic food pH increases palatability and consumption and extends drosophila lifespan. J Nutr. 145(12), 2789-2796.

Deslou-Paoli, J.M., Sornin, J.M, Heral, M. (1987). Variations saisonieres in situ de la production et de la composition des biodepots de trois mollusques estuariens (Mytilus edulis, Crassostrea gigas, Crepidula fornicata). Haliotis. 16, 2333-2345.

Devegowda, G., Arvind, B.I.R., Morton, M.G. (1996). Saccharomyces cerevisiae and mannanoligosaccharides to counteract aflatoxisis in broilers. Proc Aust Poult Sci Symp. 8, 103-106.

Dias Filho, N.L., Carmo, D.R. (2006). Study of an organically modified clay: selective adsorption of heavy metal ions and voltammetric determination of mercury(II). Talanta. 68, 919-927.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., et al. (1987). Epidemiology of Aflatoxin Formation by Aspergillus-Flavus. Annual Review of Phytopathology. 25, 249-270.

Donnay, J. D. (1952). In International Tables for X-ray Crystallography; Scheick, J. Eds.; Kynoch Press: Baltimore, Maryland.

Du, J., Feifei, L., Dai Yanhui, Z.J., Zhenyu, W. (2016). Adsorption behavior and mechanism of environmental pollutants on graphene oxide. Adv Earth Sci. 31, 1125-1136.

Duke, S.O., Baerson, S.R., Rimando, A.M. (2003). Glyphosate. Encyclopedia of Agrochemicals.

Duke, S.O., Powles SB. (2008). Glyphosate: A once-in-a-century herbicide. Pest Manag Sci. 64(4),319- 325.

Dunnlvant, F.M., Elzerman, A.W. (1992). Quantitative structure-property relationships for aqueous solubilities and henry's law constants of polychlorinated biphenyls. Environ Sci Technol. 26(8), 1567-1573.

Durfee, R.L., Contos, G., Whitmore, F.C., Barden, J.D., Hackmann, E.E. III, Westin, R.A. (1976). PCBs in the United States - industrial use and environmental distribution. Report No. EPA 56016-16-005. Springfield, VA: Versar, Inc.

E.S. Abdalla. (1997) Zearalenone: toxigenic fungi and chemical decontamination in Egyptian cereals. Nahrung, 41, 362-365.

Edition of the Drinking Water Standards and Health Advisories; (2012). United State Environmental Protection Agency: Washington, DC;

El-Aswad, A.F. (2007). Effect of organic amendments on aldicarb sorption-desorption and soil-bound residue. Journal of Applied Sciences Research. 3(11), 1437-1448.

Elmore, S.E., Mitchell, N., Mays, T., Brown, K., Marroquin-Cardona, A., et al. (2014). Common African cooking processes do not affect the aflatoxin binding efficacy of refined calcium montmorillonite clay. Food Control. 37, 27-32.

End, D., Carchman R.A., Dewey, W.L. (1979). Disruption of rat brain synaptosomal membrane by the neurotoxic insecticide Keptone. Fed Proc. 38(3), 845.

EPA (Environmental Protection Agency). (2014). Toxicological review of benzo[a]pyrene (CASRN 50-32-8) in support of summary information on the integrated risk information system (IRIS) supplemental information. Washington, DC.

EPA. (1993). Reregistration Eligibility Decision (RED): Glyphosate. U.S. Environmental Protection Agency. EPA738R93014.

Erdogan, B., Sakizci, M. (2012). Ethylene adsorption on acid-treated clay minerals. Adsorpt Sci Technol. 30(3), 265-273.

Erickson, D.C., Loehr, R.C, Neuhauser, E.F. (1993). PAH loss during bioremediation of manufactured gas plant site soil. Water Res. 27, 911-919.

Eriksson, M., Hardell, L., Carlberg, M., Akerman, M. (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int J Cancer. 123(7),1657-1663.

Etienne, M., Dourmad, J.Y. (1994). Effects of zearalenone or glucosinolates in the diet on reproduction in sows: a review. Livest Prod Sci. 99-113.

Etzel, R.A. (2002). Mycotoxins. JAMA. 287(4), 425-427.

European Food Safety Authority (2015a). Peer review report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance glyphosate.

European Food Safety Authority (2015b). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA J. 13,4302-4302.

Evaluation of the Toxicology of PCBs. (1989). United State Environmental Protection Agency: Washington, DC. https://semspub.epa.gov/work/01/54773.pdf.

Fadaei, H., Watson, A., Place, A., Connolly, J., Ghosh, U. (2015). Effect of PCB bioavailability changes in sediments on bioaccumulation in fish. Environ Sci Technol. 49(20), 12405-12413.

Fairey, J.L., Wahman, D.G., Lowry, G.V. (2010). Effects of natural organic matter on PCB-activated carbon sorption kinetics: implications for sediment capping applications. J Environ Qual. 39(4), 1359-1368.

FAO and WHO. (2016). Pesticides residues in food 2016. Special session of the Joint FAO/WHO meeting on pesticide residues. FAO plant production and protection paper. Food and Agriculture Organization of the United Nations, World Health Organization. http://www.fao.org/3/a-i5693e.pdf.

Fentabil, M., Gebremedhin, M., Purdon, J.G., Cochrane, L., Goldman, V.S. (2018). Degradation of pesticides with RSDL® (reactive skin decontamination lotion kit) lotion: LC–MS investigation. Toxicol Lett. 293, 241-248.

Food and Drugs Act. Marketing authorization for food additives that may be used as pH adjusting agents, acid-reacting materials or water correcting agents. https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/10-adjusting-agents.html

Food and Nutrition Board, Institute of Medicine, National Academy of Sciences, Subcommittee on Folate, Other B Vitamins, and Choline. (1998). Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B-6, Folate, Vitamin B-12, Pantothenic Acid, Biotin and Choline National Academy Press Washington, D.C.

Furukawa, K., Tomizuka, N., Kamibayashi, A. (1983). Metabolic breakdown of Kaneclors (polychlorobiphenyls) and their products by Acinetobacter sp. Appl Environ Microbial. 46(1), 140-145.

George, C.J., Bennett, G.F., Simoneaux, D., Geroge, W.J. (1988). Polychlorinated biphenyls a toxicological review. Journal of Hazardous Materials. 18(2), 113-114.

Germany. (2015). Final addendum to the renewal assessment report on glyphosate, compiled by efsa.

Gerritse, R.G., Beltran, J., Hernandez, F. (1996). Adsorption of atrazine, simazine, and glyphosate in soils of the Gnangara Mound, Western Australia. Aust J Soil Res. 34(4),599-607.

Geusau, A., Tschachler, E., Meixner, M., Sandermann, S., Papke, O., et al. (1999). Olestra increases faecal excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Lancet. 354(9186), 1266-1267.

Gitipour, S., Hufft, W., Bodocsi, A. (1997). The efficiency of modified bentonite clays for removal of aromatic organics from oily liquid wastes. Spill Sci Technol Bull. 4, 155-164.

Glass, R.L. (1987). Adsorption of glyphosate by soils and clay minerals. J Agric Food Chem. 35(4),497- 500.

Goes, E.H., Savage, E.P., Gibbons, G., Aaronson, M., Ford, S.A., et al. (1980). Suspected foodborne carbamate pesticide intoxications associated with ingestion of hydroponic cucumbers. Am J Epidemiol. 111, 254-259.

Grant, P.G., Phillips, T.D. (1998). Isothermal adsorption of aflatoxin B1 on HSCAS clay. J Agric Food Chem. 46(2), 599-605.

Gray, S.J., Ward, T.L., Southern, L.L. Ingram, D.R. (1998). Interactive effects of sodium bentonite and coccidiosis with monensin or salinomycin in chicks. Poult Sci. 77, 600-604.

Greenland, D.J., Quirk, J.P. (1960). Adsorption of 1-n-alkyl pyridinium bromides by montmorillonite. Clays Clay Miner. 9, 484-499.

Güler, Ç., Tunç, F. (1992). Chlorophyll adsorption on acid-activated clay. J Am Oil Chem Soc. 69, 948.

Gunawan, N.S., Indraswati, N., Ju, Y.-H., Soetaredjo, F.E., Ayucitra, A., Ismadji, S. (2010). Bentonites modified with anionic and cationic surfactants for bleaching of crude palm oil. Appl Clay Sci. 47, 462-464.

Guthrie, E.A, Pfaender, F.K. (1997). Reduced pyrene bioavailability in microbially active soil E.E. Creppy. Update of survey, regulation and toxic effects of mycotoxins in Europe

Guyton, K.Z., El Ghissassi, F., Benbrahim-Talaa, L., Grosse, Y., Loomis, D., Straif, K. (2015a). Recent progress in mechanistic data evaluation: the iarc monographs perspective. Environ Mol Mutagen. 56, S84–S84.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N. et al (2015b). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490-491.

Haines, R.G. (1971). Ingestion of aldicarb by human volunteers: a controlled study of the effect of aldicarb on man. Unpublished report with addendum. Union Carbide Corporation.

Harlow, B.L., Cramer, D.W., Bell, D.A., Welch, W.R. (1992). Perineal exposure to talc and ovarian cancer risk. Obstet Gynecol. 80(1), 19-26.

Harvey, R.B., Kubena, L.F., Phillips, T.D., Huff, W.E., Corrier, D.E. (1989). Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. Am J Vet Res. 50, 416-420.

Hensen, E.J., Smit, B. (2002). Why clays swell. J Phys Chem. 106, 12664-12667.

Hiraizumi, Y., Takahashi, M., Nishimura, H. (1979). Adsorption of polychlorinated biphenyl onto sea bed sediment, Marine Plankton, and Other Adsorbing Agents. Environ Sci Technol. 13(5), 580-584.

Hongu, N., Sachan, D.S. (2003). Carnitine and choline supplementation with exercise alter carnitine profiles, biochemical markers of fat metabolism and serum leptin concentration in healthy women. J Nutr. 133(1), 84-89.

HSDB (Hazardous Substances Data Bank). (2012). Benzo(a)pyrene [Database]. Bethesda, MD: National Library of Medicine. Retrieved from http://toxnet.nlm.nih.gov/cgi16 bin/sis/htmlgen?HSDB

https://www.epa.gov/sites/production/files/2018-03/documents/dwtable2018.pdf.

https://www.tceq.texas.gov/waterquality/tmdl/77-trinity_pcbs.html.

https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep93.pdf. April 27, 2017.

Hutzinger, Safe, S., Zitko V. The Chemistry of PCBs. CRC Press, Cleveland, Ohio, (1974). I.C.T. Nisbet, Criteria document for PCBs. Report No. PB-225 397. Washington, D.C.: U.S. Department of Commerce, 1976. R.E. Brooks, Chemical and biological destruction of PCBs, presentated at the Summer National AIChE Meeting, Cleveland, Ohio, August, 1982.

Improving water quality in Trinity river: Assessing PCBs in fish tissue. (2019). Texas Commission on Environmental Quality: Austin, TX.

International Agency for Research on Cancer (IARC). (1993). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring Substances: Food Items and Constituents, Hetercyclic Aromatic Amines and Mycotoxins. IARC Scienctific Publications no. 56.

International Agency for Research on Cancer (IARC). (2002). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Traditional Herbal Medicines, Some Mycotoxins, Napthalene and Styrene. IARC Scienctific Publications 82.

International Agency for Research on Cancer (IARC). (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum. 92,1-853.

International Agency for Research on Cancer (IARC). (2012). Chemical Agents and Related Occupations. IARC Working Group on the Evaluation of Carcinogenic Risk to Humans.

International Agency for Research on Cancer (IARC). (2015). Some organophosphate insecticides and herbicides: tetrachlorvinphos, parathion, malathion, diazinon and glyphosate. Iarc working group. Lyon, Iarc. monogr eval. Carcinog Risk Chem Hum. 112.

Integrated Risk Information System (IRIS). (1987). Glyphosate; CASRN 1071-83-6; U.S. Environmental Protection Agency: Washington, DC.

International Programme on Chemical Safety (IPCS). (1994). Glyphosate. Environmental Health Criteria 159. Geneva, Switzerland: World Health.

Jandacek, R.J., Heubi, J.E., Buckley, D.D., Khoury, J.C., Turner, W.E., et al. (2014). Reduction of the body burden of PCBs and DDE by dietary intervention in a randomized trial. J Nutr Biochem. 25(4), 483-488.

Jauhiainen, A., Rasanen, K., Sarantila, R., Nuutinen, J., Kangas, J. (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. Am Ind Hyg Assoc J. 52(2), 61-64.

Jaynes, W.F., Zartman, R.E. (2011). Aflatoxin toxicity reduction in feed by enhanced binding to surface-modified clay additives. Toxins. 3, 551-565.

Johansson, C., Tofighi, R., Tamm, C., Goldni, M., Mutti, A., Ceccatelli, S. (2006). Cell death mechanisms in AtT20 pituitary cells exposed to polychlorinated biphenyls (PCB 126 and PCB 153) and methylmercury. Toxicol Lett. 167(3), 189-190.

Jones, R.L., Marquardt, T.E. (1987). Monitoring of aldicarb residues in Long Island, New York potable wells. Arch Environ Contam Toxicol. 16, 643-647.

Juhasz A.L., Naidu, R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeter Biodegr. 45(1-2), 57-88.

Kang, S., Xing, B. (2007). Adsorption of dicarboxylic acids by clay minerals as examined by in situ ATR-FTIR and ex situ DRIFT. Langmuir. 23(13), 7024-7031.

Khan, S.U., Ivarson, K.C. (1982). Release of soil bound (nonextractable) residues by various physiological groups of micro-organisms. Environ Sci Health B. 17, 737-749.

Kiang, D.T., Kennedy, B.J., Pathre, S.V., Mirocha, C.J. (1978). Binding characteristics of zearalenone analogs to estrogen receptors. Cancer Res. 38, 3611-3615.

Kiessling, K.H., Pettersson, H., Sandholm, K., Olsen, M. (1984). Metabolism of aflatoxin, ochratoxin, zearalenone, and three tricothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. Appl Environ Microbiol. 47, 1070-1073.

Klick, M.A., Pitt, J.I. (1988). Differentiation of Aspergillus flavus from A. parasiticus and other closely related species. Trans Br Mycol Soc. 91, 99-108.

Komadel, P., Schmidt, D., Madejova, J., Cicel, B. (1990). Alteration of smectites by treatments with hydrochloric acid and sodium carbonate solutions. App Clay Sci. 5(2), 113-122.

Koskinen, W.C, Harper, S.S. (1990). The retention processes: mechanisms H.H Chang (Ed.), Pesticides in the soil environment: processess, impacts and modelling, soil science society of america, Madisin, WI, 51-77.

Kouadio, J.H., Mobio, T.A., Baudrimont, I., Moukha, S., Dano, S.D., Creppy, E.E. (2005). Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. Toxicology. 213, 56-65. doi: 10.1016/j.tox.2005.05.010

Kuiper-Goodman, T., Scott, P.M., Watanabe, H. (1987). Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharmacol. 7, 253-306. doi: 10.1016/0273-2300(87)90037-7.

Larson, P.S., Egle, J.L., Jr., Hennigar, G.R., Lane, R.W. Bqrzelleca, J.F. (1979). Acute, sub- chronic, and chronic toxicity of chlordecone. Toxicol Appl Pharmacol. 48, 49.

Lehman, A.J. Summaries of Pesticide Toxicity. The Association of Food and Drug Officials of the United States, Business Office, Topeka, Kansas, 1965. V. Zitko and P.M.K. Choi, PCBs and other industrial halogenated hydrocarbons in the environment. Technical Report No. 272, St. Andrews, New Brunswick, Canada: Fisheries Research Board of Canada, 1971.

Lemke, S.L., Grant, P.G., Phillips, T.D. (1998). Adsorption of zearalenone by organophilic montmorillonite clay. J Agric Food Chem. 46(9), 3789-3796.

Li, Y., Tian, G., Dong, G., Bai, S., Han, X., Liang, J., Meng, J., Zhang, H. (2018). Research progress on the raw and modified montmorillonites as adsorbents for mycotoxins: A review. Appl Clay Sci. 163, 299-311.

Lioi, M.B., Santoro, A., Barbieri, Salzano, R., Ursini, M.V. (2004). Ochratoxin and zearalenone: a comparative study on genotoxic effects and cell death induced in bovine lymphocytes. Mutat. Res. 557(1), 19-27.

Liu, C., Gu, C., Yu, K., Li, H., Teppen, B.J., Johnston, C.T., Boyd, S.A., Zhou, D. (2015). Integrating Structural and Thermodynamic Mechanisms for Sorption of PCBs by Montmorillonite. Environ Sci Technol. 49(5), 2796-2805.

Liu, P., Zhang, L. (2007). Adsorption of dyes from aqueous solutions or suspensions with clay nano-adsorbents. Sep Purif Technol. 58, 32-39.

Loffredo, E., Castellana, G., Senesi, N. (2014). Decontamination of a municipal landfill leachate from endocrine disruptors using a combined sorption/bioremoval approach. Environ Sci Pollut Res Int. 21(4), 2654-2662.

Lohmann, R., MacFarlane, J., Gschwend, P. (2005). Importance of black carbon to sorption of native PAHs, PCBs, and PCDDs in Boston and New York harbor sediments. Environ Sci Technol. 39, 141-148.

Luongo, D., Severino, L., Bergamo, P., De Luna, R., Lucisano, A., Rossi, M. (2006). Interactive effects of fumonisin B1 and alpha-zearalenol on proliferation and cytokine expression in Jurkat T cells. Toxicol In Vitro. 20, 1403-1410.

Madejova, J., Bujdak, J., Janek, M., Komadel, P. (1998). Comparative FT-IR study of structural modifications during acid treatment of dioctahedral smectites and hectorite. Spectrochim Acta Part A, Mol Biomol Spectrosc. 54(10), 1397-1406.

Maki, C.R., Allen, S., Wang. M., Ward, S.H., Rude, B.J., Bailey, H.R., Harvey, R.B., Phillips, T.D. (2017). Calcium Montmorillonite Clay for the Reduction of Aflatoxin Residues in Milk and Dairy Products. JDVS. 2(3), 555587.

Maki, C.R., Thomas, A.D., Elmore, S.E., Romoser, A.A., Harvey, R.B., Ramirez-Ramirez, H.A., and Phillips, T.D. (2016). Effects of calcium montmorillonite clay and aflatoxin exposure on dry matter intake, milk production, and milk composition. JDS. 99, 1039-1046.

Marketing authorization for food additives that may be used as pH adjusting agents, acid-reacting materials or water correcting agents, https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted.

Marroquin-Cardona, A., Deng, Y., Garcia-Mazcorro, J., Johnson, N.M., Mitchell, N., et al. (2011). Characterization and safety of uniform particle size NovaSil clay as a potential aflatoxin enterosorbent. Appl Clay Sci. 54(3-4), 248-257.

Mayes, B.A., McConnell, E.E., Neal, B.H., Brunner, M.J., Hamilton, S.B., et al. (1998). Comparative Carcinogenicity in Sprague-Dawley Rats of the Polychlorinated Biphenyl Mixtures Aroclors 1016, 1242, 1254, and 1260. Toxicol Sci. 41, 62-76.

Mazumder, P.M., Sasmal, D. (2001). Mycotoxins - limits and regulations. Anc Sci Life. 20(3), 1-19.

McDonough, K.M., Fairey, J.L., Lowry, G.V. (2008). Adsorption of polychlorinated biphenyls to activated carbon: equilibrium isotherms and a preliminary assessment of the effect of dissolved organic matter and biofilm loadings. Water Res. 42(3), 575-584.

McKenzie, K.S., Sarr, A.B., Mayura, K., Bailey, R.H., Millar, D.R., et al. (1997). Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. Food Chem Toxicol. 35, 807-820.

Meador, J.P., Stein, J.E, Reichert, W.L, Varanasi, U. (1995). Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Rev Environ Contam Toxicol. 143, 79-165.

Megharaj, M., Garthwaite, I., Thiele, J.H. (1997). Total biodegradation of the oestrogenic mycotoxin zearalenone by a bacterial culture. Lett App Microbiol. 24, 329-333.

Mehmood, Z., Smith, A.G., Tucker, M.J., Chuzel, F., Carmichael, N.G. (2000). The development of methods for assessing the in vivo oestrogen-like effects of xenobiotics in CD-1 mice. Food Chem Toxicol. 38, 493-501. doi: 10.1016/S0278-6915(00)00022-3

Merliss, R.R. (1971). Talc-treated rice and Japanese stomach cancer. Science. 173(4002), 1141-1142.

Metcalf, R.L., Sanbom, J.R., PO-Yang, L., Nye, D. (1975). Laboratory model ecosystem studies of the degradation and fate of radiolabeled tri-, tetra-, and pentachlorobiphenyl compared with DDE. Arch Environ Contam Toxicol. 3, 151-165.

Meylan, W., Howard, P., Boethling, R. (1996). Improved Method for Estimating Water Solubility from Octanol/water Partition Coefficient. Environ Toxicol Chem. 15, 100-106.

Miles, C.J., Moye, H.A. (1988). Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils. J Agric Food Chem. 36(3),486-491.

Miller, D.M., Stuart, B.P., Crowell, W.A. (1981). Experimental aflatoxicosis in swine: morphological and clinical pathological results. Can J Comp Med. 45(4), 343-351.

Minervini, F., Giannoccaro, A., Cavallini, A., Visconti, A. (2005). Investigations on cellular proliferation induced by zearalenone and its derivatives in relation to the estrogenic parameters. Toxicol Lett. 159, 272-283.

Mitchell, N.J., Xue, K.S., Lin, S., Marroquin-Cardona, A., Brown, K.A., et al. (2014) Calcium montmorillonite clay reduces AFB1 and FB1 biomarkers in rats exposed to single and co-exposures of aflatoxin and fumonisin. JAT. 34, 795-804.

Mocchegiani, E., Corradi, A., Santarelli, L., Tibaldi, A., DeAngelis, E., et al. (1998). Zinc, thymic endocrine activity and mitogen responsiveness (PHA) in piglets exposed to maternal aflatoxicosis B1 and G1. Vet Immunol Immunopathol. 62(3), 245-260.

Mohammad-Khah, A., Ansari, R. (2009). Activated charcoal: preparation, characterization and applications: A review article. Int J Chemtech Res. 1(4), 859-864.

Mokaya, R., Jone, W., Daies, M.E., Whittle, M.E. The mechanism of chlorophyll adsorption on acid-activated clays. J Solide State Chem. 111(1), 157-163.

Mokoena, M.P., Chelule, P.K., Gqaleni, N. (2005). Reduction of fumonisin B1 and zearalenone by lactic acid bacteria in fermented maize meal. J Food Prot. 68, 2095-2099.

Monson, M.S., Coulombe, R.A., Reed, K.M. (2015). Aflatoxicosis: Lessons from toxicity and responses to aflatoxin B1 in poultry. Agriculture. 5, 742-777.

Morillo, E., Undabeytia, T., Maqueda, C., Ramos, A. (2000). Glyphosate adsorption on soils of different characteristics. Influence of copper addition. Chemosphere, 40(1), 103-107.

Mortland, M.M., Sun, S., Boyd, S.M. (1986) Clay-organic complexes as adsorbents for phenol and chlorophenols. Clays Clay Miner. 34, 581-585.

Mueller, S.O., Simon, S., Chae, K., Metzler, M., Korach, K.S. (2004). Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. Toxicol Sci. 80, 14-25. doi: 10.1093/toxsci/kfh147

Mullin, M., Sawka, G., Safe, L., McCrindle, S., Safe, S. (1981). Synthesis of the octa- and nonachlorobiphenyl isomers and congeners and their quantitation commercial polychlorinated biphenyls and identification in human breast milk. J Anal Toxicol. 5(3), 138-142.

Mullins, M.D., Pochini, C.V., McCrindle, S., Romkes, M., Safe, S.H., Safe, L.M. (1984). High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners. ES&T. 18(6), 468-476.

Murugesan, G.R., Ledoux, D.R., Naehrer, K., Berthiller, F., Applegate, T.J., et al. (2015). Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci. 94, 1298-1315.

National Academy of Sciences. (1977). Drinking Water and Health. Washington, DC. 635-643.

National Registration Authority. (2001). The NRA review of aldicarb. National Registration Authority for Agricultural and Veterinary Chemicals: Canberra, Austrilia.

Ncibi, M.C., Sillanpää, M. (2015). Optimized removal of antibiotic drugs from aqueous solutions using single, double and multi-walled carbon nanotubes. J Hazard Mater. 298, 102-110.

Neiger, R., Johnson, T., Hurley, D., Higgins, K., Rottinghaus, G., et al. (1994). The short-term effect of low concentrations of dietary aflatoxin and T-2 toxin on mallard ducklings. Avian diseases. 738-743.

Neji, S.B., Trabelsi, M., Frikha, M.H. (2011). Esterification of fatty acid over Tunisian acid activated clay: kinetic study. J Oleo Sci. 60(6), 293-299.

Nestle, M. (1998). The selling of olestra. Public Health Rep. 113(6), 508-520.

Newman, L.M., Johnson, E.M., Giacobbe, R.L., Fu, L.J. (1990). The in vitro activation of cyclophosphamide in the hydra developmental toxicology assay. Toxicol Sci. 15, 488-499.

Nikov, G.N., Hopkins, N.E., Boue, S., Alworth, W.L. (2000). Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor-estrogen response element complex formation. Environ Health Perspect. 108, 867-872. doi: 10.1289/ehp.00108867

Oh, S., Im, H., Oh, E., Lee, J. et al. (2004). Effects of benzo(a)pyrene on protein expression in Jurkat T-cells. Proteomics, 4, 3514-3526.

Olsen, M., Pettersson, H., Sandholm, K., Visconti, A., Kiessling, K.H. (1987). Metabolism of zearalenone by sow intestinal mucosa in vitro. Food Chem Toxicol. 25, 681-683. doi: 10.1016/0278-6915(87)90101-3

Onishi, H.A., Trench, W.C. (1981). PCBs in perspective. Ind Wastes. 39-35.

Ontario Ministry of the Environment. (1985). Review of benzo[a]pyrene. Occurrence, human exposure and health effects. Prepared by Canviro Consultants Ltd., Kitchener and Toronto.

Osborne, M.R., Crosby, N.T. (1987). Benzopyrenes. Cambridge Monographs on Cancer Research. Cambridge, England: Cambridge University Press.

Ottinger, S.E., Mayura, K., Lemke, S.L., McKenzie, K.S., Wang, N., Kubena, L.F., Phillips, T.D. (1999). Utilization of electrochemically generated ozone in the degradation and detoxification of benzo[a]pyrene. J Toxicol Environ Health Part A. 57(8), 565-583. DOI: 10.1080/009841099157511.

Ozcan, A.S., Ozcan, A. (2004). Adsorption of acid dyes from aqueous solutions onto acid-activated bentonite. J Colloid Interface Sci., 267(1), 39-46.

Perreault, F., de Faria, A.F., Elimelech, M. (2015). Environmental applications of graphene-based nanomaterials. Chem Soc Rev. 44, 5861-5896.

Philip, J.W., Gates, W.P., Patti, A.F., Scott, J.L. (2011). Catalytic activity of choline modified Fe(III) montmorillonite. Appl Clay Sci. 53(2), 336-340.

Phillips, T.D., Kubena, L.F. Harvey, R.B., Taylor, D.R., Heidelbaugh, N.D. (1987). Mycotoxin hazards in agriculture: New approach to control. J Am Vet Med Assoc. 190, 1617 (Abstract).

Phillips, T.D., Kubena, L.F. Harvey, R.B., Taylor, D.R., Heidelbaugh, N.D. (1988). Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. Poult Sci. 67, 243-247.

Phillips, T.D. (1999). Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicol Sci. 52, 118-126.

Phillips, T.D., Afriyie-Gyawu, E., Williams, J., Huebner, H., Ankrah, N.A., et al. (2008). Reducing human exposure to aflatoxin through the use of clay: A review. Food Addit Contam Part A. 25(2), 134-45.

Phillips, T.D., Sarr, A.B., Grant, P.G. (1995). Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. Nat Toxins. 3(4), 204-213, discussion 221.

Phillips, T.D., Wang, M., Elmore, S.E., Hearon, S., Wang, J.S. (2019). NovaSil clay for the protection of humans and animals from aflatoxins and other contaminants. Clay. Clay Miner. 67(1), 99-110. https://doi.org/10.1007/s42860-019-0008-x.

Piccolo, A., Celano, G., Arienzo, M., et al. (1994). Adsorption and desorption of glyphosate in some European soils. J Environ Sci Health Part B. 29(6),1105-1115.

Pier, A. (1985). In utero transfer of aflatoxin and selected effects in neonatl pigs. Trichothecenes and other mycotoxins. J. Lacey. New York, Wiley, 495-506.

Pignatello, J.J., Xing, B. (1996). Mechanisms of slow sorption of organic chemicals to natural particles. Environ Sci Technol. 30, 1-11.

Plimmer, J.R., Bradow, J.M., Dionigi, C.P., et al. (2004). Herbicides. In: Kirk-Othmer encyclopedia of chemical technology. John Wiley & Sons, Inc.

Pollock, B.H., Elmore, S., Romoser, A., Tang, L., Kang, M.S., et al. (2016). Intervention trial with calcium montmorillonite clay in a south Texas population exposed to aflatoxin. Food Addit Contam Part A. Chem Anal Control Expo Risk Assess. 33(8), 1346-1354. DOI: 10.1080/19440049.2016.1198498.

Radian, A., Mishael, Y. (2012). Effect of humic acid on pyrene removal from water by polycation-clay mineral composites and activated carbon. Environ Sci Technol. 46, 6228-6235.

Rajagopalan, R., Vohra, K.G., Monhan Rao, A.M. (1983). Studies on oxidation of benzo[a]pyrene by sunlight and ozone. Sci Total Environ. 27, 33-42.

Rajaie, S., Esmaillzadeh, A. (2011). Dietary choline and betaine intakes and risk of cardiovascular diseases: review of epidemiological evidence. ARYA atherosclerosis. 7, 78-86.

Rajaie, S., Esmaillzadeh, A. (2011). Dietary choline and betaine intakes and risk of cardiovascular diseases: review of epidemiological evidence. ARYA Atherosclerosis. 7, 78-86. Richardson, S.M., Richardson, J.M., 1982. Crystal Structure of a Pink Muscovite from Archer's Post, Kenya: Implications for Reverse Plechorism in Dioctahedral Micas. Am Miner. 67, 69-75.

Ramesh, A., Inyang, F., Hood, D.B., Archibong, A.E., et al. (2001). Metabolism, bioavailability, and toxicokinetics of benzo([alpha])pyrene in F-344 rats following oral administration. Exp Toxicol Pathol. 53, 275-290.

Ramos, A.J., Hernandez, E., Pla-Delfina, J.M., Merino, M. (1996). Intestinal absorption of zearalenone and in vitro study of non-nutritive sorbent materials. Int J Pharm. 128, 129-137.

Resmi, G., Thampi, S.G., Chandrakaran, S. (2012). Removal of lead from wastewater by adsorption using acid-activated clay. Environ Technol. 33(1-3), 291-297.

Rhodes, C.N., Brown, D.R. (1993). Surface properties and porosities of silica and acid-treated montmorillonite catalyst supports: influence on activities of supported ZnCl2 alkylation catalysts. J Chem Soc. 89, 1387-1391.

Ricci, K.A., Girosi, F., Tarr, P.I., Lim, Y.W., Mason, C., et al. (2006). Reducing stunting among children: the potential contribution of diagnostics. Nature. 444 (Suppl 1), 29-38.

Richardson, S.M., Richardson, J.M. (1982). Crystal structure of a pink muscovite from archer's post, Kenya: Implications for reverse plechorism in dioctahedral micas. Am Miner. 67, 69-75.

Risher, J.F., Mink, F.L., Stara, J.F. (1987). The toxicologic effects of the carbamate insecticide aldicarb in mammals: A review. Environ Health Perspect. 72, 267-281.

Rodriguez, R.R., Basta, N.T., Casteel, S.W., Pace, L.W. (1999). An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. Environ Sci Technol. 33, 642-649.

Rogers, A.E. (1993). Nutritional modulation of aflatoxin carcinogenesis. The toxicology of aflatoxins: human, health, veterinary, and agricultural significance. D. L. Eaton and J. D. Groopman. London, Academic Press.

Rotenberg, B., Patel, A.J., Chandler, D. (2011). Molecular explanation for why talc surfaces can be both hydrophilic and hydrophobic. Acc Chem Res. 133(50), 20521-20527.

Rowe, J.N. (1983). Relative source contributions of diet and air to ingested asbestos exposure. Environ Health Perspect. 53, 115-120.

Safe, S.H. (1994). Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol. 24(2), 87-149.

Sanders, T.H., Blankenship, P.D., Cole, R.J., Hill, R.A. (1984). Effect of soil temperature and drought on peanut pod and stem temperatures relative to Aspergillus flavus invasion and aflatoxin contamination. Mycopathologia. 86(1), 51-54.

Sarr, A.B., Mayura, K., Kubena, L.F., Harvey, R.B., Phillips, T.D. (1995). Effects of phyllosilicate clay on the metabolic profile of aflatoxin B1 in Fischer-344 rats. Toxicol Lett. 75, 145-151.

Schulze, D., Dixon J., Weed, S. (1989). Minerals in soil environments. Minerals in soil environments.

Sexton W.F. (1966). Report on aldicarb.

Shushkova, T., Ermakova, I., Leontievsky, A. (2010). Glyphosate availability in soil. Biodegradation. 21, 403-410.

Slade, P.G., Raupach, M., Emerson, W.W. (1978). The Ordering of Cetylpyridinium Bromide on Vermiculite. Clays Clay Miner. 26, 125-134.

Smith, E.A., Oehme, F.W. (1992). The biological activity of glyphosate to plants and animals: A literature review. Vet Hum Toxicol. 34(6),531-543.

Smith, W.E. (1973). Asbestos, talc and nitrites in relation to gastric cancer. Am Ind Hyg Assoc J. 34(5), 227-228.

Sprankle, P., Meggitt, W.F., Penner, D. (1975). Rapid inactivation of glyphosate in the soil. Weed Sci. 23(3), 224-228.

Sun, Y., Yang, S., Zhao, G., Wang, Q., Wang, X. (2013). Adsorption of polycyclic aromatic hydrocarbons on graphene oxides and reduced graphene oxides. Chem Asian J. 8, 2755-2761.

Swithers, S.E., Ogden, S.B., Davidson, T.L. (2011). Fat substitutes promote weight gain in rats consuming high-fat diets. Behav Neurosci. 125(4), 512-518.

Tapp, H., Stotzky, G. (1995). Insecticidal activity of the toxins from bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and soil clays. Appl Environ Microbiol. 61(5), 1786-1790.

Tarasevich, Y.I., Aksenenko, E.V. (2014). Hydrophobicity of talc basal surface. Colloid J. 76(4), 483-489.

Taylor, J.F., Phillips T.D. (2010). Ferrihydrite as an enterosorbent for arsenic. Toxicol Lett. 127(2002), 19-28.

Turner, P.C. (2013). The molecular epidemiology of chronic aflatoxin driven impaired child growth. Scientifica (Cairo). 152879.

Tyagi, B., Chudasama, C.D., Jasra, R.V. (2006). Determination of structural modification in acid activated montmorillonite clay by FT-IR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 64, 273-278. DOI: 10.1016/j.saa.2005.07.018.

U.S. Environmental Protection Agency Website. Summary assessment of the results of sampling of localized areas identified for focused investigations following Hurricane Katrina. https://archive.epa.gov/katrina/web/html/focused_sampling.html/

U.S. Food and Drug Administration. Food Additive Status List. https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm091048.htm

Ugochukwu, U. C., Fialips, C. I. (2017). Crude oil polycyclic aromatic hydrocarbons removal via clay-microbe-oil interactions: Effect of acid activated clay minerals. Chemosphere. 178, 65-72. DOI: 10.1016/j.chemosphere.2017.03.035.

Ullah, Z., Hussain, S., Gul, S., Khan, S., Bangash, F.K. (2015). Use of HCl-modified bentonite clay for the adsorption of acid blue 129 from aqueous solutions. Desalin Water Treat. 57(19), 8894-8903.

United States Environmental Protection Agency. (2011). Toxicological review of benzo[a]pyrene (CAS No. 50-32-8). In Support of Summary Information on the Integrated Risk Information System.

United States Environmental Protection Agency. (2016). Glyphosate Issue Paper: Evaluation of Carcinogenic Potential. EPA's Office of Pesticide Programs.

US Department of Commerce, National Technical Information Service. (1979). Polychlorinated Biphenyls, Committee on the Assessment of Polychlorinated BiphenyIs in the Environment, Washington, D.C.

Van Noort, P.C., Jonker, M.T., Koelmans, A.A. (2004). Modeling maximum adsorption capacities of soot and soot-like materials for PAHs and PCBs. Environ Sci Technol. 38, 3305-3309.

Vidal, E., Negro, A., Cassano, A., Zalazar, C. (2015). Simplified reaction kinetics, models and experiments for glyphosate degradation in water by the UV/H2O2 process Photochem Photobiol Sci. 14, 366-377.

Wang, M., Hearon, S., Phillips T.D. (2019). Development of enterosorbents that can be added to food and water to reduce toxin exposures during disasters. J Environ Sci Health B. 54(6), 1-11.

Wang, M., Hearon, S.E., Johnson, N.M., Phillips, T.D. (2019). Development of broad-acting clays for the tight adsorption of benzo[a]pyrene and aldicarb. Appl Clay Sci. 168, 196-202.

Wang, M., Maki, C.R., Deng, Y., Tian, Y., Phillips, T.D. (2017). Development of high capacity enterosorbents for aflatoxin B1 and other hazardous chemicals. Chem Res Toxicol. 30(9), 1694-1701. DOI: 10.1021/acs.chemrestox.7b00154.

Weissenfels, W.D, Klewer, H., Landhoff, J. (1992). Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: influence on biodegradability and biotoxicity. Appl Microbiol Biotechnol. 36, 689-696.

Wilby, O.K., Tesh, J.M., Shore, P.R. (1990). Application of the hydra regeneration assay: Assessment of the potential teratogenic activity of engine exhaust emissions. Toxicol In Vitro. 4(4-5), 612-613.

Wiles, M.C., Huebner, H.J., McDonald, T.J., Donnelly, K.C., Phillips, T.D. (2005). Matrix-immobilized organoclay for the sorption of polycyclic aromatic hydrocarbons and pentachlorophenol from groundwater. Chemosphere. 59, 1455-1464.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., et al. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am J Clin Nutr. 80(5), 1106-1122.

Wolf-Fordham, S.B., Twyman, J.S., Hamad, C.D. (2014). Educating first responders to provide emergency services to individuals with disabilities. Disaster Med Public Health Prep. 8(6), 533-40. doi: 10.1017/dmp.2014.129.

Wong, C., Hempling, R.E., Piver, M.S., Natarajan, N., Mettlin, C.J. (1999). Perineal talc exposure and subsequent epithelial ovarian cancer: a case-control study. Obstet. Gynecol. 93, 372-376.

Wong, J., Hu, W., Downward, G.S., Seow, W.J., Bassig, B.A., et al. (2017). Personal exposure to fine particulate matter and benzo[a]pyrene from indoor air pollution and leukocyte mitochondrial DNA copy number in rural China. Carcinogenesis. 38(9), 893-899.

World Health Organization. (1984). Guidelines for drinking water quality. Vol. 3. Geneva. 183-189.

Wright, A.S., Donniger, C., Greenland, R.D., Stemmer K.L., Zavon, M.R. (1978). The effects of prolonged ingestion of die&in on the livers of male rhesus monkeys. Ecotox Environ Safe. 1(4), 477.

Wu, W., Chen, W., Lin, D., Yang, K. (2012). Influence of surface oxidation of multiwalled carbon nanotubes on the adsorption affinity and capacity of polar and nonpolar organic compounds in aqueous phase. Environ Sci Technol. 46, 5446-5454.

Xu, G., McMahan, C.A., Walter, C.A. (2014). Early-life exposure to benzo[a]pyrene increases mutant frequency in spermatogenic cells in adulthood. PloS One. 9(1).

Yang, K., Zhu, L., Xing, B. (2006). Adsorption of polycyclic aromatic hydrocarbons by carbon nanomaterials. Environ Sci Technol. 40, 1855-1861.

Yarsan, E., Tanyuksel, M., Celik, S., Aydin, A. (1999). Effects of aldicarb and malathion on lipid peroxidation. Bull Environ Contam Toxicol. 63(5), 575-581.

Yip, A.C., Lam, F.L., Hu, X. (2005). A novel heterogeneous acid-activated clay supported copper catalyst for the photobleaching and degradation of textile organic pollutant using photo-Fenton-like reaction. Chem Commun. 25, 3218-3220. DOI: 10.1039/b501531f.

Yonei, Y., Takahashi, Y., Hibino, S., Watanabe, M., Yoshioka, T. (2008). Effects on the human body of a dietary supplement containing L-carnitine and garcinia cambogia extract: A study using double-blind Tests. J Clin Biochem Nutr. 42, 89-103.

Zedeck, M.S. (1980). Polycyclic aromatic hydrocarbons, a review. J Environ Pathol Toxicol. 3, 537.

Zeisel, S.H., Blusztajn, J.K. (1994). Choline and human nutrition. Ann Rev Nutr. 14,269-296.

Zeisel, S.H., da Costa, K.-A., Franklin, P.D., Alexander, E.A., Lamont, J.T., et al. (1991). Choline, an essential nutrient for humans. FASEB J. 5,2093-2098.

Zhang, G., Feng, Y., Song, J., Zhou, X. (2018). Zearalenone: A mycotoxin with different toxic effect in domestic and laboratory animals' granulosa cells. Front Genet. 9, 667.

Zhang, H.M., Nie, J.-S., Wang, F., Shi, Y.-T. et al. (2008). Effects of benzo[a]pyrene on autonomic nervous system of coke oven workers. J Occup Health. 50, 308-316.

Zhang, R., Gao, H., Ren, Z., Cen, D., Chen, Z. (2017). Preparation of activated bentonite and its adsorption behavior on oil-soluble green pigment. Physicochem Probl MI. 53(2), 829-845.

Zhou H., George, S., Hay, C., Lee, J., Qian, H., Sun, X. (2017). Individual and combined effects of Aflatoxin B1, Deoxynivalenol and Zearalenone on HepG2 and RAW 264.7 cell lines. Food Chem Toxicol. 103,18-27.

Zimmerman, J.R., Ghosh, U., Millward, R.N., Bridges, T.S., Luthy, R.G. (2004). Addition of carbon sorbents to reduce PCB and PAH bioavailability in marine sediments: physicochemical tests. Environ Sci Technol. 38(20), 5458-5464.